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Contribuições para decifrar a trajetória da Doença Pulmonar Obstrutiva Crónica: associações entre medidas genéticas e clínicas

Contributions for unravelling Chronic Obstructive Pulmonary Disease trajectory: associations between genetics and clinical measures

Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biomedicina Molecular, realizada sob a orientação científica da Doutora Alda Sofia Pires de Dias Marques, Professora Adjunta da Escola Superior de Saúde da Universidade de Aveiro e da Doutora Gabriela Maria Ferreira Ribeiro de Moura, Professora Auxiliar do Departamento de Ciências Médicas.

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palavras-chave

doença pulmonar obstrutiva crónica, DPOC, gene, polimorfismo de nucleótido simples, medidas reportadas pelos pacientes, medidas clínicas.

resumo

A Doença Pulmonar Obstrutiva Crónica (DPOC) é uma doença multifatorial e heterogénea que apresenta impactos diferentes em pacientes com estadios da doença semelhantes. Isto sugere que outros fatores para além da função pulmonar podem afetar a forma como o paciente experiencia a sua doença. As medidas reportadas pelo paciente (PROs) são um conjunto de variáveis que permitem avaliar a autoperceção e experiência dos doentes em relação à sua doença. Estudos recentes reportaram a existência de associações entre polimorfismos de nucleótidos simples (SNPs) e PROs, mas pouco ainda se sabe sobre estas associações e seu significado. Assim, este estudo teve como objetivo principal explorar possíveis associações entre variantes genéticas específicas e medidas clínicas, entre as quais PROs. Pretendeu também contribuir para a caracterização dos genótipos dos doentes com DPOC em Portugal.

Realizou-se um estudo transversal com 60 doentes com DPOC. As PROs avaliadas foram 1) frequência de exacerbações autoreportadas pelos doentes, 2) dispneia com a modified medical Research Council Scale e Borg, 3) fadiga com a Borg, 4) ansiedade e depressão através da escala de ansiedade e depressão hospitalar, 5) impacto da doença com o teste de avaliação da DPOC e 6) qualidade de vida relacionada com a saúde (QVRS) com questionário do hospital de St. George na doença respiratória. Adicionalmente, outras medidas clínicas também foram avaliadas, i. é., função pulmonar, força muscular periférica com dinamometria digital, força dos músculos respiratórios com a medição das pressões respiratórias e capacidade funcional através do teste de levantar e sentar cinco vezes e 1 minuto. Zaragatoas orofaríngeas e amostras de saliva foram recolhidas de todos os pacientes para genotipagem.

Foram encontradas associações significativas entre variantes genéticas e dispneia (rs1143634, rs1042717, rs1138272 e rs12504628), fadiga (rs1042714 e rs1138272), ansiedade (rs1051303, rs1800450 e rs1131620), impacto da doença (rs10461985 e rs1172113) e QVRS (rs11172113, rs1042713, rs1138272 e rs12504628). Também foram encontradas variantes significativamente associadas à função pulmonar (rs1042713, rs1042717 e rs5030737), força dos músculos respiratórios (rs1130866), força muscular periférica (rs1042713, rs1042717, rs11172113 e rs11556218) e capacidade funcional (rs12899618, rs11046966 e rs1138272).

Este foi um estudo exploratório e mais investigações são necessárias para confirmar os resultados obtidos e para explorar mais profundamente a associação e interpretação entre a genética e a trajetória da DPOC.

keywords

chronic obstructive pulmonary disease, COPD, gene, single nucleotide polymorphism, patient-reported outcomes, clinical outcomes.

abstract

Chronic Obstructive Pulmonary Disease (COPD) is a multifactorial and heterogeneous disease which impacts differently on patients with similar grades. This suggests that factor others than lung function may affect patients experience of the disease. Patient-reported outcomes (PROs) are a set of measures that allow to assess patients' self-perception and experience of the disease. Recent studies have reported associations between specific single nucleotide polymorphisms (SNPs) and PROs, however not much is known about these associations and their meanings. Thus, this study had as main objective to explore possible associations between specific genetic variants and clinical measures, including PROs. It also sought to contribute for a characterization of the genotypes from patients with COPD in Portugal.

A cross-sectional study was conducted in a total number of 60 patients with COPD. The PROs assessed were: 1) self-reported frequency of exacerbations, 2) dyspnoea with modified Medical Research Council and Borg scales , 3) fatigue with Borg scale, 4) anxiety and depression with Hospital Anxiety and Depression scale; 5) impact of the disease with COPD Assessment Test and 6) health-related quality of life (HRQOL) with St. George Respiratory questionnaire; Additionally, several surrogate outcomes were also assessed i. e., lung function, peripheral muscle strength with digital dynamometer, respiratory muscle strength with the respiratory pressure assessment and functional capacity through the 1 minute and 5 time sit-to-stand. Both oropharyngeal swabs and saliva samples were collected from the patients for genotyping.

Significant associations were found between genetic variants and dyspnoea (rs1143634, rs1042717, rs1138272 and rs12504628), fatigue (rs1042714, rs1138272), anxiety (rs1051303, rs1800450 and rs1131620), impact of the disease (rs10461985 and rs11172113) and HRQOL (rs11172113, rs1042713, rs1138272 and rs12504628). Significant associations were also found between genetic variants and lung function (rs1042713, rs1042717, rs5030737), respiratory muscle strength (rs1130866), peripheral muscle strength (rs1042713, rs1042717, rs11172113, rs11556218) and functional capacity (rs12899618, rs11046966 and rs1138272).

This was an exploratory study and more investigations are necessary to confirm the results obtained and to explore deeply the associations and interpretations between genetics and COPD trajectory.

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5STS	Five time seat to stand
AAT	Alpha 1 antitrypsin
ADRB2	*Beta 2-adrenergic receptor*
BMI	Body Max Index
CAT	Chronic Obstructive Pulmonary Disease Test
COPD	Chronic Obstructive Pulmonary Disease
ESSUA	*Escola Superior de Saúde da Universidade de Aveiro*
FEV_1pp	Forced expiratory volume in one second percent predicted
FVCpp	Forced vital capacity percent predicted
GOLD	Global Initiative for Chronic Obstructive Pulmonary Disease
IBIMED	Instituto de Biomedicina da Universidade de Aveiro
LRP1	LDL receptor related protein1
LTBP4	*Latent transforming growth factor beta binding protein 4*
MBL2	*Mannose binding lectine 2*
MEP	Maximum expiratory pressure
MIP	Maximum inspiratory pressure
mMRC	Modified Medical Research Council
PROM	Patient-reported outcome measure
PROs	Patient-reported outcomes
SFTPB	Surfactant Protein B

SNPs	Single nucleotide polymorphisms
SpO_2%	Peripheral oxygen saturation
STS	Sit to stand
USA	United States of America

1. INTRODUCTION

Chronic obstructive Pulmonary Disease (COPD) is a major public health problem (1) known to affect 210 million people worldwide(2) and 800.000 in Portugal (3). By 2020 it will be the 3rd leading cause of mortality accounting already for 3.2 million deaths in 2015(4). Given the substantial and growing economic costs, ascending a more than 38.6 billion of Euros in Europe and 49.9 billion in the USA(5), but also the social burden, significant research efforts have been made in the last decade to enhance our understanding of COPD development, diagnosis, impact and management.

The risk of developing COPD is usually associated to an interaction between genetics and environmental factors(1). Genetic factors have been associated with risk for COPD such as the deficiency of α1-antytripsine (AAT)(6). Nevertheless, this deficiency only affects 1/3 of all COPD cases, thus there has been successive efforts to find other candidate genes that may also be a risk for COPD(7). In the last years, hundreds of studies were conducted in an attempt to find genetic variants in candidate genes associated to the disease pathogenesis, susceptibility and progression. However, these studies have mainly focus on associating these variants with lung function (main criteria for establishing COPD diagnosis), neglecting the impact of this condition on patients. Patient-reported outcomes (PROs) are a set of measures about patients' health status which are directly reported by the patient. They provide the real perception of the impact of the disease on patients' life and guide meaningful interventions(8). Nevertheless, associations between genetic variants and PROs have been poorly investigated, as demonstrated in our systematic review conducted within the scope of this dissertation and currently under consideration in PLOSone (Appendix I). If this patient-centered information was to be integrated, a significant knowledge advance would certainly be achieved on our understanding of COPD development, diagnosis and management.

Although significant number of international studies have been conducted to characterise the genetic of these patients, in Portugal, the genetics of COPD is a theme yet to be decoded. Nevertheless, one study has been conducted in Madeira Island to assess the prevalence of the AAT genotypes in young males between 18 and 23 years old and consequently risk for COPD(9).

Therefore, this study aimed to explore associations between specific genetic variants and several clinical measures, some of which PROs of COPD. Additionally, it was also aimed to contribute with an exploratory study for the genotype characterisation of patients with COPD in Portugal.

Here, the introduction of this dissertation is presented (chapter 1). Chapter 2 provides a deeper knowledge to the state of the art and is followed by chapter 3 which describes the settings where patients with COPD were contacted and recruited as well as all measures collected and the laboratorial and clinical procedures carried out. Chapter 4 presents the results obtained in the clinical and genetic fields. Finally, chapter 5 presents the discussion about the results obtained and chapter 6 the conclusions and future implications.

2. STATE OF THE ART

2.1. Brief overview

Chronic obstructive pulmonary disease (COPD) is one of the deadliest pathologies worldwide. Defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) as "common, preventable and treatable disease that is characterized by persistent respiratory symptoms and airflow limitation"(1), COPD accounts for 3.2 million deaths in 2015, which represents an increase of 12% since 1990 (4). It is currently estimated that the observed increase in risk factors such as, aging of the populations (10), and emissions of air pollutants(11), may increase the mortality numbers to 6 millions deaths by 2020(12). In Portugal this disease has a known prevalence of 14%(13), and killed 2365 people in 2013 which corresponded to 20,04% of all deaths caused by respiratory diseases in Portugal(14).

The high prevalence, mortality and morbidity of COPD, makes this disease a major health, economic and social burden. Worldwide between one and thirty thousand dollars are spent every year per patient(15). A substantial portion of these expenses are due to exacerbations, i.e.," acute worsening of respiratory symptoms that results in additional therapy"(1) . Therefore, early identification of those at risk of developing COPD is nowadays, together with COPD diagnosis and management, a top priority for research.

2.2. Risk Factors

The risk of developing COPD is usually associated to an interaction between genetics and environmental factors(1). Smoking is considered the environmental risk factor most commonly associated with COPD(1). In fact, it has been shown that cigarette smokers usually present more airflow limitations and other abnormalities than non or ex-smokers(16). However, only a small fraction (20%) of smokers develop the disease(7), which suggests that other factors may be involved and contribute to the risk of developing COPD.

Genetic factors have also been considered as a risk for COPD such as the deficiency of α1-antytripsine (AAT)(6). This serine protease plays an important role in the inhibition of the functions of neutrophil elastase in the lungs, but polymorphisms responsible for decreasing the concentrations of AAT in plasma when combined with risk factors may lead to emphysema(17). Nevertheless, this deficiency only affects 1-3% of all COPD cases, thus there has been successive efforts to find other candidate genetic variants with relevance for COPD(7).

Exposure to outdoor and indoor air pollution is also a risk factor to develop COPD(7). It has been shown that exposure to toxicity of particles from outdoor air may play a heavy role in

increasing the number of exacerbations in patients with COPD(18). Moreover, the fact that most people spend more hours indoors, makes indoor pollution (vapours, fireplaces) a higher risk factor to develop COPD(1, 7, 19).

Other risk factors that have been associated to COPD are ageing and gender, asthma, infections in early life, and socioeconomic problems(7). Figure 1 summarises the identified risk factors for developing COPD.

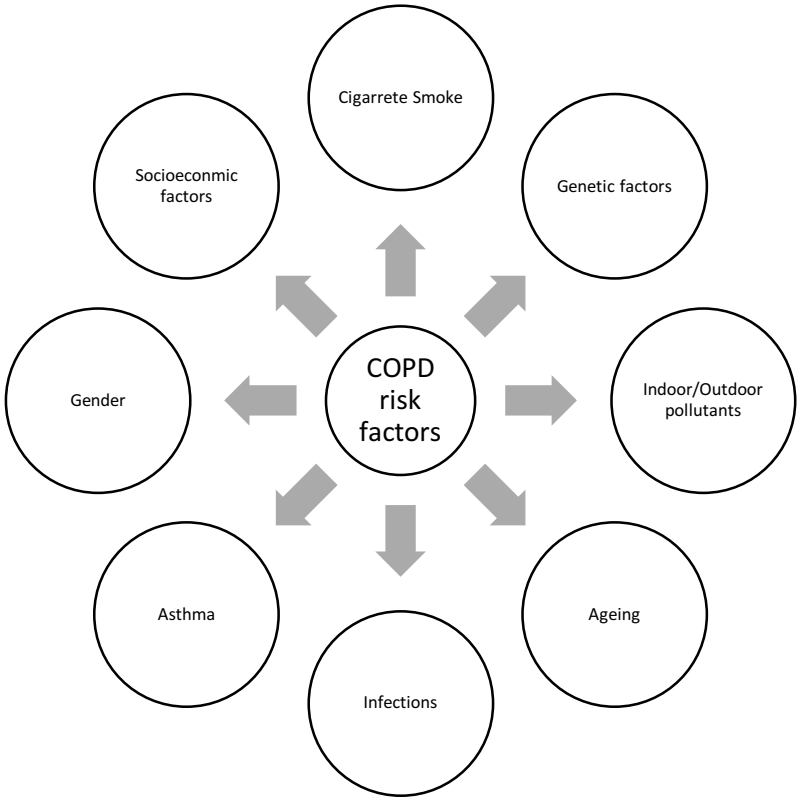


Figure 1- Risk factors for developing Chronic Obstructive Pulmonary. Disease.

2.3. Chronic Obstructive Pulmonary Disease and its clinical manifestations

Patients with COPD usually present symptoms as cough, dyspnoea, increased sputum production and wheezing(20). Cough may be the only symptom present in patients and also may be more prominent in the morning. Furthermore, cough is usually accompanied by sputum that usually vary in amount(21). Dyspnoea, which refers to the sensation of breathlessness, is one of the most important and commonly observed symptoms in patients with COPD(22). This symptoms is usually associated with limited physical activity, which may lead to anxious and depressive behaviours(22). Wheezing, is usually described as a noisy breathing and vary between days(21). Additionally, since COPD shares risk factors with other pathologies may lead to a coexistence of this disease with other comorbidities as cardiovascular diseases, obstructive sleep apnoea, diabetes, cachexia or osteoporosis, which may lead to onset of other symptoms (23).

2.4. Disease classification

According to GOLD guidelines(1), diagnosis and assessment of the disease is based on a lung function test, usually performed with spirometry which assesses forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC). In conjunction with patients' clinical history, symptoms and exposure to risk factors history assessment, a post-bronchodilator FEV_1/FVC inferior to 0.70 may represent a diagnosis of COPD(20).

In previous versions of GOLD (1), some cut-of values from spirometry test were used as a method to assess the severity of airflow obstruction, which has been divided in four categories (Table 1).

Table 1-Global Initiative for Chronic Obstructive Pulmonary Disease classification of airflow limitation severity. Adapted from [1]

GOLD 1	Mild	$FEV_1 \geq 80$ predicted
GOLD 2	Moderate	$50\% \leq FEV_1 < 80\%$ predicted
GOLD 3	Severe	$30\% \leq FEV_1 < 50\%$ predicted
GOLD 4	Very severe	$FEV_1 < 30\%$ predicted

Recently, a new classification for COPD severity was proposed. In addition to the GOLD classification of airflow severity previously referred (GOLD 1,2,3,4), this new classification also takes into account patients-reported outcomes (PROs), i.e., the modified Medical Research Council Dyspnoea Scale (mMRC) and COPD Assessment test (CAT). Thus, four new categories were created: A (patients with mMRC between 0 and 1, CAT score <10 and 0 or 1 exacerbation that not lead to hospital admission), B (patients with mMRC≥2, CAT≥10 and 0 or 1 exacerbation that not lead to hospital admission), C (patients with mMRC between 0 and 1, CAT<10 and ≥2 or ≥1 exacerbation that leads to hospital admission) and D (patients with mMRC≥2, CAT≥10 and ≥2 or ≥1 exacerbation that leads to hospital admission) (Figure 2)(1).

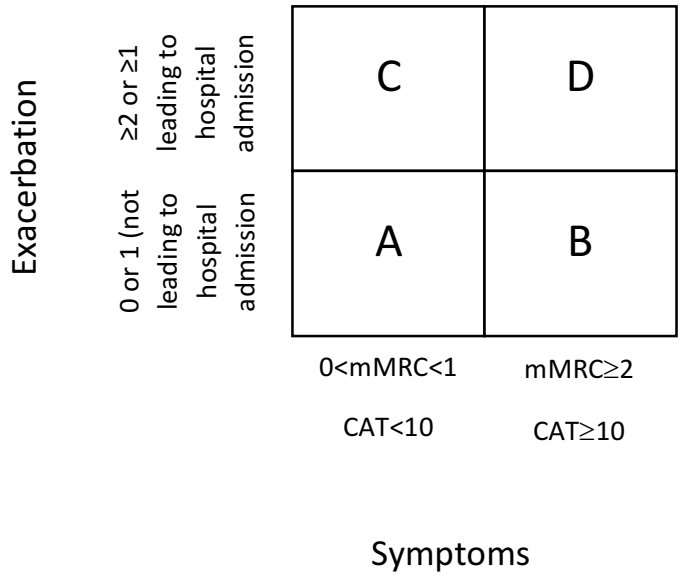


Figure 2-The Global Initiative for Chronic Obstructive Lung Diseases ABCD assessment. Adapted from (1).

2.5. Patient-reported outcomes

PROs are a set of measures about patients' health status which are directly reported by the patient(8).

Since the insertion of the new ABCD disease classification based on PROs, a new attention and importance has been given to these outcomes. COPD is a heterogeneous disease, that impacts not only at a pulmonary level, but also at a extrapulmonary level (e.g., muscle strength, balance, quality of life, emotional well-being, among many others)(24). FEV_1 is one of the most common lung function parameters to assess disease progression and to evaluate treatments, however FEV_1 does

not correlate with changes in symptoms, COPD comorbidities or respective consequences that may lead to a decline in the quality of life (24, 25). Additionally, patients with similar FEV_1 values may have different experiences of the disease(25). Thus, the inclusion of PROs measures (PROM) is fundamental to assess patient's perception and progression of the disease adequately and consequently adapt treatments according to their specific needs.

There are already a significant number of valid, reliable and responsive PROM used to assess outcomes of interest as health-related quality of life (HRQOL), self-reported exercise capacity and frequency of exacerbations among others. Additionally, other PROs are being proposed under the scope of COPD which, although some have not yet been validated, do show the increased awareness for this type of measurements to better assess patients with this disease (Figure 3)(24, 25).

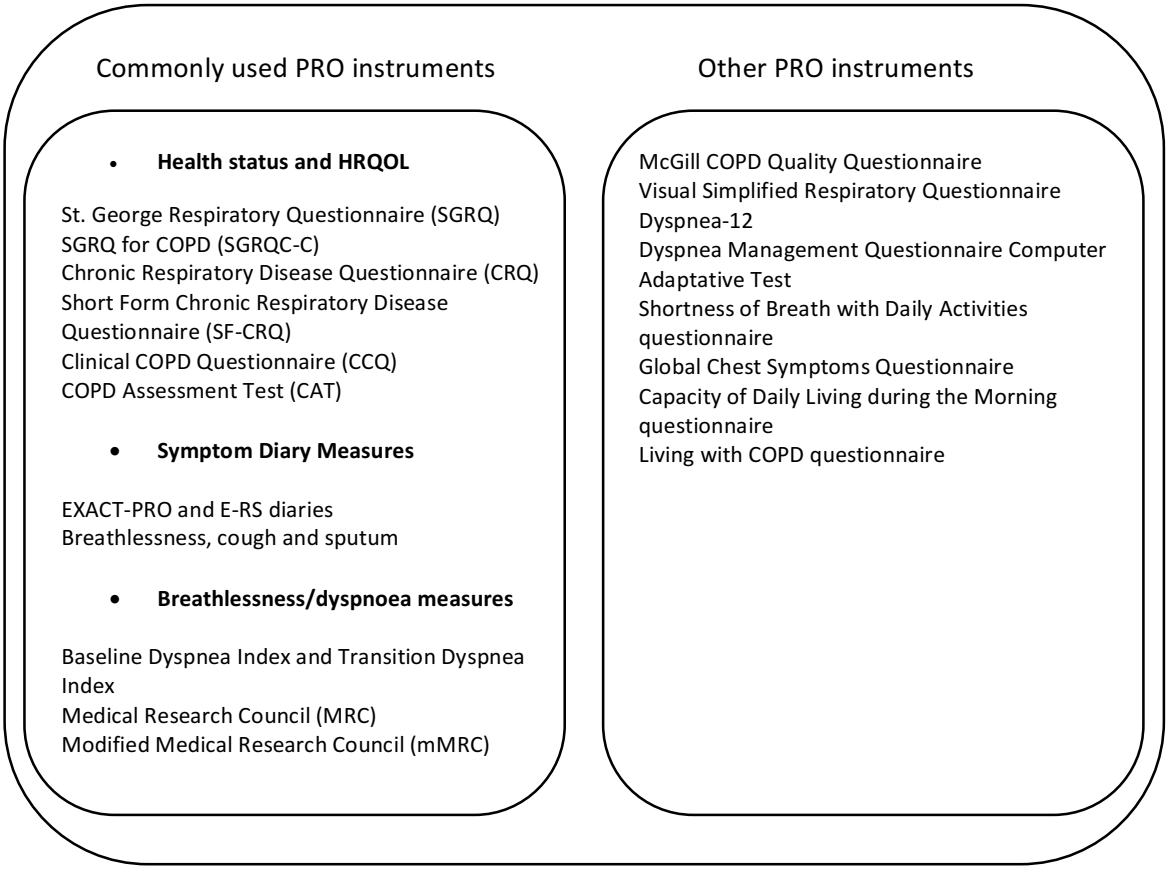


Figure 3-Commonly used patient-reported outcomes measures in Chronic Obstructive Pulmonary Disease and other patient-reported outcomes used. Adapted from (25).

2.6. Genetics of Chronic Obstructive Pulmonary Disease

COPD is a multifactorial disease, caused by an association of alleles found in multiple genes, environmental factors and interactions between these two factors. Since the discovery of AAT variants (more than 57 years ago) as a risk factor for COPD, the role of genetics has gained a new attention to understand the onset and evolution of this disease(26).

In the last years, hundreds of studies were performed in an attempt to find genetic variants in candidate genes associated to the disease pathogenesis, susceptibility and progression.

Single nucleotide polymorphisms (SNPs) are the exchange of single bases at specific positions and are one of the genetic variants most common and studied relative to any pathology. In fact, more than four hundred SNPs in two hundred genes were already associated with COPD in the literature, in different genome-wide association studies (GWAS) (for further information, see genetic variants table in Appendix II). Additionally, with the appearance of DNA genotyping arrays, these genetic variants may be measured more economically and in greater quantity. An array is a container object that may be composed of thousands of specific short fragments of DNA (oligonucleotides) which hybridize with sequences of target DNA being analysed, allowing to genotype thousands of SNPs simultaneously in different patients(27).

Despite all this progress, there are still several problems associated with the genetics of this disease. First, controversial results were being obtained among studies investigating the same specific genetic variant(28). This may be explained by the different populations from different ethnicities being studied, use of small sample sizes or different environments among populations (29, 30). Second, all the efforts of the genetic studies have been concentrated on finding associations between specific genetic variants with lung function or with smoking to enhance our understanding of the susceptibility to the disease(6). However, the association between genetic variants and other important outcomes such as PROs, which provide the real impact and progression of the disease via patients' perception have been poorly investigated. This has been the theme of our systematic review currently under consideration in PLOSone, which demonstrates the exiguity of data relating genetic factors with PROs in COPD (Appendix I).

Therefore, research is needed at a worldwide level to integrate all the important information provided and to enhance our understanding on COPD development, diagnosis and management. In Portugal, the genetics of COPD is a theme yet to be decoded, since only one study was conducted in Madeira Island to assess the prevalence of the AAT genotypes in young males between 18 and 23 years old and consequently risk for COPD(9).

2.7. Patient-reported outcomes and genetics

COPD is without doubts a complex and heterogeneous disease, with different morphological manifestations, different comorbidities, that may impact differently on patients at similar grades and may have implications for disease progression and management(31). Thus, personalized and tailored interventions are needed for these patients based not only on patients attributes, but also on biomarkers and other variables(32).

In the last years, it has become more evident that there is a strong association between PROs and genetics at different fields(33, 34). PROs are important mainly to assess conditions that only patients may report as pain, fatigue, depression or anxiety however, they are also important to understand the biological pathways associated with this condition(32). In the respiratory field, more precisely in lung cancer, associations between PROs and specific genetic variations have already been demonstrated (35). Thus, by knowing which genetic variant is associated with each PRO, professionals will be able to identify patients genotype that makes them more susceptible to a PRO. With this information, tailored intervention may be applied, which can contribute positively for increasing patients' quality of life and behavioural changes, resulting in a significant reduction in health care system expenditures (32).

2.8. Objectives

The main goal of this masters' dissertation was to explore associations between specific genetic variants and several clinical measures some of which PROs in patients with COPD. This will allow a better understanding of the susceptibility of these patients to the PROs deficits, predict the disease progression and later inform the development of tailored interventions. Additionally, this dissertation also aimed to contribute for the genetic characterisation of patients with COPD in Portugal, since this theme is still poorly explored.

3. METHODS

This study is part of a larger study entitle "*GENIAL – Marcadores genéticos e clínicos na trajetória da DPOC"*, funded by Programa Operacional de Competitividade e Internacionalização - COMPETE, through Fundo Europeu de Desenvolvimento Regional - FEDER (POCI-01-0145-FEDER-016701), Fundação para a Ciência e Tecnologia (PTDC/DTPPIC/2284/2014) and under the project UID/BIM/04501/2013.

3.1. Ethics

Ethical approval was previously obtained from Administração Regional de Saúde Centro (ARS-Centro) (64/2016), from Centro Hospitalar do Baixo Vouga (CHBV) (08-03-17), Hospital Pedro Hispano (10/CE/JAS) and Hospital Distrital da Figueira da Foz. Documents may be consulted at Annex I. Prior to any data collection, written informed consents were collected from patients.

3.2. Design and Participants

A cross-sectional study was conducted in the north and center region of Portugal. Hospitals and Primary Care Centres of Baixo Vouga and Baixo Mondego, Hospital Pedro Hispano and Hospital Distrital da Figueira da Foz were contacted, from which three hospitals and two primary care centres accepted to participate. Patients were eligible if i) diagnosed with COPD according to GOLD criteria(1) ii) presented in a stable state, i.e., no acute exacerbations in the previous month, iii) were able to give informed consent. Exclusion criteria were the presence of severe cardiac, musculoskeletal or neuromuscular diseases, cognitive impairment or a history of neoplasia or immune disease that would interfere with patients' collaboration in data collection or interpretation. Patients with COPD were recruited from the emergency and internal medicine services of the Centro Hospitalar do Baixo Vouga, Hospital Pedro Hispano, Hospital Distrital da Figueira da Foz or by contacting patients previously informed by the referred institutions. In the hospital services, the physician informed each potential participant about the study. Only patients that showed interest to participate were approached by the researcher to provide more information about the study and ask about their willingness to participate. Patients recruited through telephone calls were informed about the study and if they agreed to participate, they were integrated as stable patients with COPD (stCOPD).

3.3. Measures

A structured questionnaire was first used to collect socio-demographic (name, gender, date of birth, address, academic qualifications, marital status and occupation), anthropometric (height, weight, percentage of body fat and body mass index [BMI]) and clinical (smoking habits, comorbidities, medication, home oxygen or ventilation supplies, dyspnoea, hospital admission in the past 3 months/year, exacerbations in the past year and physical activity levels) data in order to characterise the population. This questionnaire was followed by several other measures that are described below.

Dyspnoea. This parameter was assessed using mMRC, a reliable, valid and responsive questionnaire to assess activity limitations due to dyspnoea in patients with chronic respiratory diseases, namely COPD(36, 37). It is composed of 5 statements on a scale from 0 to 4, with higher scores indicating higher respiratory limitation(38).

Dyspnoea and fatigue. The modified Borg scale is a valid, reliable(39) and commonly used tool to assess dyspnoea and fatigue at rest in patients with COPD(40, 41). This is a 0 to 10 rated scale, where the patient points his/her perception of dyspnoea/fatigue(42). The modified Borg scale allows a score interpretation, in which 0 corresponds to an absence of dyspnoea or fatigue and 10 corresponds to patient's maximum sensation of dyspnoea/fatigue, existing also intermediate options according to patients' perception of the symptoms(43).

Anxiety and depression. These parameters were assessed using the Portuguese version of the Hospital Anxiety and Depression Scale (HADS)(44). This is reported as a valid and reliable instrument and is commonly used in patients with COPD(45, 46). This scale contains 14 questions, seven questions measuring symptoms of anxiety (HADS-A) and 7 measuring symptoms of depression (HADS-D). Each question has four possible answers ranged from 0 to 3, so the possible scores ranged from 0 (patient has no depression or anxiety) to 21 (maximum depression/anxiety reported by the patient). Scores inferior to 8 were considered "normal values", between 8 and 10 were interpreted as "mild values", between 11 and 14 as "moderate values" and between 15 and 21 as "severe values" of anxiety and depression symptoms(44).

Impact of COPD. To assess the disease impact on well-being and daily life of participants, the CAT was used(47). This is a simple, reliable, responsiveness and validated test in COPD(47, 48). This scale is composed of 8 questions with 6 options each numbered from 0 to 5 with a maximum score of 40, that intends to explore the impact of COPD on patient's health status, mainly daily symptoms

and other manifestations of the disease(49). Higher scores indicate higher impact of the disease on patients' life.

Health-related Quality of life. St. George Respiratory Questionnaire (SGRQ)(50) was used to assess health-related quality of life in patients with COPD. This tool is validated and has been consistently used in patients with COPD (51-53). It is composed of 76 items divided in three domains, which measure symptoms, the impact of disease and the activity limitations. The score ranges from 0 to 100, with higher scores indicating poor health-related quality of life (54).

Vital signs and oxygen saturation. In order to establish a baseline and monitor the patient, both vital signs and oxygen saturation (SpO_2%) were initially assessed. Blood pressure was verified using a portable automatic sphygmomanometer which also provided the heart rate (Medel Elite, S.Polo di Torrile, Italy), SpO_2% was monitored using a pulse oximeter (Konica Minolta, Pulsox-300i, United Kingdom), and the respiratory rate was assessed using a stopwatch to monitor the number of respiratory cycles taken in one minute.

Lung function. This was assessed by spirometry (MicroLab 3500, CareFusion, Kent, United Kingdom), a simple and non-invasive test that may identify the presence of obstructive respiratory abnormalities(55). It is valid, reliable and commonly used in the COPD population(56). It was performed according to the current guidelines(57).

Peripheral muscle strength. Muscle dysfunction and poor exercise capacity is one of the biggest concerns in COPD(58). For that reason, quadriceps muscle strength was assessed with a hand-held dynamometer (Hoggan MicroFET2 Muscle Tester, Model 7477, Pro Med Products, Atlanta, GA). This test, previously used in patient with COPD (59), is valid and reliable(60) to assess lower-extremity muscle strength in older adults, similar to the study population. Additionally, the isometric handgrip force was measured with a hydraulic-hand dynamometer (Model 12-0241 Lite, Fabrication Enterprises Inc., White Plains, NY, USA). This is a valid measurement of mobility and quality of life in patients(61).

Respiratory muscle strength. Measurement of static maximum inspiratory pressure (MIP) and maximum expiratory (MEP) pressures are commonly performed in patients with COPD (62, 63) to assess inspiratory/expiratory muscle strength, since respiratory (specially inspiratory) muscle function is frequently altered in these patients(62). MIP and MEP were assessed using respiratory pressure gauge (MicroRPM, CareFusion, Kent, United Kingdom).

Functional capacity. Five times sit-to-stand test (5STS) and 1-minute sit-to-stand (STS) were used to assess functional capacity. 5STS and STS test are a reliable, valid and responsive test in patients with COPD. Both tests are commonly used in COPD patients to determine functional state with less stress for the patient comparing with other tests as the 6 minutes walking test for example(64).

Genetic. Oropharyngeal swabs and falcons were used to collect buccal detached cells and saliva respectively.

3.4. Procedures

Assessment sessions took place at the School of Health Sciences, University of Aveiro (ESSUA) in the Respiratory Research and Rehabilitation Laboratory (Lab3R), participating hospitals or at patient's home. Initially, a brief description of the study purpose was provided by the researcher and time was given for the participant to read the information sheet, clarify any doubts and sign the informed consent. Then, a code was assigned to each participant, to guarantee the confidentiality of the participation, and socio-demographic, anthropometric and clinical data were collected to characterise the population. Sequentially, questionnaires were completed according to the guidelines and always under supervision of the researcher.

Vital signs and SpO_2 were measured and dyspnoea/fatigue were quantified using modified Borg scale and mMRC.

Participant's lung function was then assessed (57), and the most frequently measurements considered in spirometry: FVC, FEV_1 and FEV_1/FVC ratio were registered(55).

Quadriceps muscle strength was assessed based on O'shea protocol (59), where patients were asked to sit on a chair with the knee flexed 70°. The hand-held dynamometer was placed in the anterior tibia region, 5 cm above the lateral malleolus from the dominant lower limb and the patient was instructed to stretch the knee over a 4-second period against the resistance applied (Figure 4). This process was repeated 3 times with a recovery period of 30 seconds to avoid fatigue and the best result was recorded(59).

Figure 4- Test of quadriceps muscle strength using hand-held dynamometer.

Additionally, the isometric handgrip force was also assessed according to international recommendations(65).Strength was assessed at the dominant hand with the elbow 90º flexion. Three attempts were performed and the highest value was taken.

Respiratory muscle strength assessment was performed as previously described (62), with some minor alterations. In a first step, all the procedure was demonstrated to the patient using a spare mouthpiece. Starting with MIP, firstly patients were instructed to sit on a chair in the upright position. Then a nasal clip was placed in patients' nose to guarantee that there was no air lost through the nasal passages. After this, with the mouth out of the rubber buccal, patients were asked to expire as slowly as possible and when there was no more air to expire, patients were instructed to put their mouth in the buccal and inspire as fast and intense as possible. MEP was exactly the reverse process. Once again with a nasal clip in patients' nose, they were asked to inspire in a slow and relaxed way with the mouth outside of the buccal. When there was no more air to inspire, patients were instructed to put the buccal in the mouth and to expire as fast as possible (Figure 5). For both cases, three trials were performed with rest periods of 30 seconds between trials. The best MIP and MEP values from the three trials were registered.

Figure 5- Initial position of the maximal inspiratory/expiratory pressure.

Functional muscle strength was then assessed with the 5STS and 1 minute STS (66). Patients were asked to sit on a chair (floor to seat height 48 cm) well stabilized against the wall and with upper limbs across the chest and knees and hips flexed to 90°. Then patients were asked to stand up and sit down 5 times as fast as possible with the arms crossed on their chest. The duration of each test was timed with a stopwatch, that started on the command "go", and stopped at the fifth sit-to-stand repetition. 1 minute STS was performed as reported for 5STS but with the arms along the body, and in this test the stopwatch started on the command "go", the patient performed the manoeuvre sit-to-stand-to-sit as fast as possible for 1 minute. Time for rest was provided between repetitions and tests to restore basal vital signs, SpO_2%, dyspnoea and fatigue values according to participant's need in both tests.

Oropharyngeal swabs and falcons with saliva were collected from each participant in order to extract DNA for genetic analysis. For both cases, specific precautions were taken during this procedure to avoid contamination of the samples. Patients were advised to avoid ingestion of any acidic substances, aliments rich in sugar or in caffeine, before data collection since these can cause changes in the saliva pH and lead to increased bacterial development. Patients were also advised to mouthwash with water 10 minutes before the sample collection to minimise the presence of food particles and any modification in saliva pH(67). Additional care was taken to avoid the swab touching in any other surface other than the patient's mouth, to prevent any contamination. Swabs were scrapped inside the participant's mouth during 30 seconds with rotational movements, taking into account the care referred above(67) and patients were asked to spit several times into the

falcons during the entire protocol. Once finished the collection, the swabs and falcons were properly labelled, transported in portable freezers to the Institute for Research in Biomedicine (iBiMED) where were preserved in freezers at -80ºC.

3.5. Data analysis

3.5.1. Sample analysis

3.5.1.1 DNA extraction

Prior to manipulation, samples were unfrozen at room temperature. Total DNA was extracted using QIAamp DNA mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions, with slight modifications.

Before the beginning of the extraction, all samples and reagents were at room temperature. First, 20 µl of Qiagen proteinase K was pipetted into two 1.5 ml microtubes. Then 200 µl of saliva was added followed by 200 µl of buffer AL to lysis cells. To ensure an efficient lysis, both microtubes with the samples and buffer AL were mixed by vortexing for 30 seconds and incubated at 56 °C for 10 minutes. After incubation, samples were briefly spinned to remove drops produced by the condensation. In order to precipitate DNA, 200 µl of 100% ethanol were added to both microtubes followed by a 15 seconds vortexing and spinning to remove droplets. Afterwards, the mixture of both microtubes was carefully applied in a QIAamp Mini spin column. The column was washed with both washing buffers: AW1 and AW2. Finally, the DNA was eluted with 200 µl of milli Q water, incubated at room temperature (15-25 °C) for 10 minutes to assure a efficient elution and the final extract was obtained by centrifugation at 6000 g for 1 minute. All samples were stored at -20 °C.

3.5.1.2. DNA quantification

The concentration of DNA samples was measured by two methods, absorbance and a fluorimetric method. Absorbance measurements were performed using Denovix DS-11 FX+ spectrophotometer. 1 µl of mili Q water was used as a blank. The same volume of sample used to obtain concentration and absorbance ratios values (A260/A280 and A260/A230).

To ensure an accurate quantification process, the amount of recovered DNA was quantified by using a Qubit dsDNA BR assay kit (Life technologies), a fluorimetric method, according to the manufacturer's instructions (Life technologies Cat# Q32850, Q32853). First, to obtain the optimal performance of the reagents, these were placed at room temperature before the beginning of the process. Qubit working solution was prepared by diluting 1 µl of Qubit dsDNA BR reagent in 199 µl of Qubit dsDNA BR buffer for each sample/standard. Each standard and sample requires 190 µl and 199 µl of Qubit working solution respectively so, enough Qubit working solution must be prepared for 2 standards and the number of samples to be analysed.

To prepare the two standards, 190 µl of Qubit working solution was added to each tube. Then 10 µl of each Qubit standard was added to both tubes followed by a vortex during 3 seconds. This process was carefully conducted to prevent the creation of bubbles that may influence the process.

Each sample was prepared adding 199 µl of Qubit working solution and 1 µl of the previously vortexed sample. Both tubes (samples) were vortexed by 3 seconds, and then were incubated at room temperature for 2 minutes to allow the Qubit assay to reach optimal fluorescence.

The Broad Range Assay programme was chosen and reading process started with Qubit fluorometer calibration. In this phase standard 1 and standard 2 were read and a line corresponding to the curve-fitting algorithm was constructed. Then, each sample was read and the corresponding yields values were registered.

3.5.1.3. DNA integrity

In order to assess the DNA samples integrity and degradation, a 1% agarose gel was conducted based on Lee et al(68).

In a first step 0.5 g of agarose was placed in a flask containing 50 ml of TAE 1× running buffer. Then, to dissolve the mixture was heated until the agarose was totally dissolved. To detect nucleic acids in the agarose gel, 3 µl of GreenSafe Premium was added to mixture. This nucleic acid stain emits green fluorescence(68) when bounded to DNA. Finally, the agarose gel was placed to cool during 10 minutes before being placed in the gel tray previously levelled.

In order to allow a visual tracking of DNA migration during electrophoresis, loading dye 6× was added to the samples. Running buffer TAE 1× was added until the surface of the gel was

covered and the loading process was ready to be started. The first well was carefully loaded with GeneRuler 1kb DNA Ladder, which is composed of DNA fragments ranging from 250 to 10 000 bp to allow for DNA sizing. Samples were slowly loaded in the other wells.

With the sample loading process completed, the lid was closed and the leads of the gel box were attached to the power supply programmed with a voltage of 120 V and the gel was run for 30 minutes.

Images of the final gel were obtained using a GelDoc XR+ Imaging System (Bio-Rad Laboratories, Hercules, CA).

3.5.1.4. DNA samples SpeedVac

DNA samples extracted were concentrated in a speed vacuum (ScanSpeed MaxiVac, Labogene, Denmark),in order to achieve the concentration values requested for the genotyping analysis institute (minimum sample concentration of 60 ng/µl; A260/280 ratio between 1.8 and 2; A260/230 ratio between 1.7 and 2). Each sample was placed on the speed vacuum rotor in counter balanced positions with the lid open. Then, the rotor was programmed for 30 minutes. Temperature and rotation led to water evaporation resulting in a less diluted DNA. However, to avoid sample degradation, at the end of 30 minutes, samples were placed on ice for 10 minutes, and then the process was repeated. Finally, DNA concentration and A260/280; A260/230 ratios were measured using DenoVix D-11.

3.5.1.5. Array genotyping

To provide a comprehensive understanding of which specific genetic variants were significantly associated with PROs, a systematic review was conducted (Appendix I). Additionally, it was important to find all the genetic variants associated with COPD in the literature and whether of these may also be associated with PROs . Thus, a targeted gene sequencing panel (Appendix II) was constructed based on a databases review.

Since the DNA microarray technology is not yet available at iBiMED, we decided to use the Infinium Global Screening Array-24 v1.0 from Illumina (Illumina, San Diego, CA, USA). This is an array mainly used for population studies where genotyping is required and, therefore, is expected to produce a good genetic stratification of our patients, that can be later connected to COPD

outcomes. To run these samples, we have chosen the laboratory of Life & Brain GmbH institute, Germany. This laboratory has plenty experience on the array and has been recommended by the Illumina dealer in Portugal (ILC, Portugal).

According to the respective literature, Infinium Global Screening array was based on Illumina Infinium HTS Assay protocol, which is composed of nine steps: 1) DNA amplification; 2) DNA incubation; 3) DNA fragmentation; 4) DNA precipitation; 5) DNA resuspension; 6) Hybridiation to BeadChip; 7) BeadChip washing; 8) Extend and stain the BeadChip and 9) Image BeadChip. In the first stage, samples were denaturated with sodium hydroxide and neutralized with the reagent "Multi-amplification 2 mix", followed by an incubation (step 2) with a time range of 20/24 hours at 37ºC. In this step, genomic DNA was amplified, resulting in a sufficient quantity of DNA to the entire process. Then, an enzymatic-based fragmentation occurred using a fragmentation solution. To avoid an excess of fragmentation, an end-point was used. DNA was then precipitated with isopropanol. DNA is less soluble in solutions with isopropanol, facilitating the precipitation process(69). Finally, samples were resuspended in hybridization and washing buffer (step 6), genomic DNA was dispensed onto BeadChips. The loaded BeadChip was incubated overnight until efficient annealing. In the next phase, the BeadChip was washed to remove non-connected or unhybridized primers (step 7) and was prepared to be stained and extended (step 8). In this last step, detectable labels were incorporated on the BeadChip. Finally, with the micro-array Hi-scan, a laser was used to excite the fluorophore, and to read the different colours, according to the probe.

All information was provided as files from the GenomeStudio software (Illumina, San Diego, CA, USA), which included all the basic information about the genotyping conditions (replication errors, call frequencies) and the genotypes and genotypes frequencies for each SNP for all the population.

3.5.2. Statistical analysis

A code was attributed to each participant and data was analyzed anonymously.

Data was analyzed using IBM Statistics version 23 (SPSS Inc., Chicago, Illinois, IL). Descriptive statistics was used to describe socio-demographic and clinical outcomes. Continuous data (age, spirometry test, MIP/MEP, quadriceps muscle strength, handgrip, and 5STS) was described as mean ± standard deviation, ordinal data (frequency of exacerbations, mMRC, dyspnoea and fatigue scores from Borg scale, anxiety and depression scores from HADS, CAT scores and 1 minute STS results)

was described as median and interquartile range and categorical data (gender, Gold classification and smoking status) was described as percentages and frequencies.

One-way ANOVA test was used to compare mean values of continuous measures (spirometry, SGRQ, MIP, MEP, quadriceps muscle strength, handgrip muscle strength, 5STS) between genotypes of each SNP assessed. When significant differences were obtained, Bonferroni correction test was used for multiple comparisons with a level of significance of $p<0.05$.

Kruskal Wallis non-parametric test was used to compare ordinal/categorical non-normally distributed data among groups (frequency of exacerbations, mMRC, Borg scale, HADS, CAT and 1 minute STS). When significant differences were verified, Mann-Whitney U test was used for multiple comparisons. Bonferroni corrections were applied for the number of comparisons performed per SNP (i.e., 3 comparisons) between outcomes. Thus, the level of significance was set at 0.017.

Genotypes characteristics (genotype frequencies, call frequencies and number of calls) were assessed using GenomeStudio software (Illumina, San Diego, CA, USA) and graphs were plotted in GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA).

4. RESULTS

4.1. Sample recruitment

Eighty-two patients with COPD from five institutions were included in the study. Twenty-two patients were excluded due to sample impurities (e.g., food particles, excess of sputum, lipstick) and consequent difficulties in DNA extraction. A total of sixty patients with COPD were enrolled in this study (Figure 6).

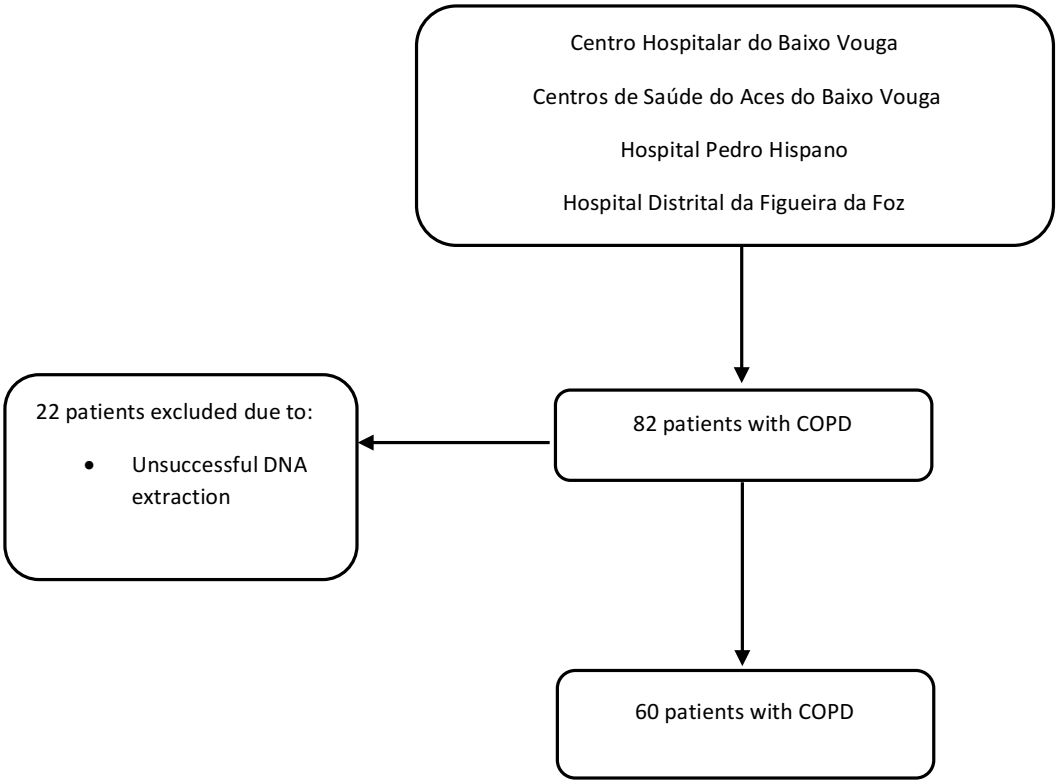


Figure 6-Sample recruitment process.

4.2. Sample characterization

Patients were mostly male (n=52) and ex-smokers (n=42) with a mean age of 67.87 (± 8.59) years old. Eleven patients were classified as GOLD A, twenty-two as GOLD B, six as GOLD C and twenty-one as GOLD D. A range of PROs and clinical outcomes (lung function, muscular and function tests) were assessed and global scores may be consulted in table 2.

Table 2-Sample characterisation.

Characteristics	**Patients with COPD (n=60)**
• Age (years)	67.87±8.59
• Gender, n (%)	
Masculine	52 (86.7)
Feminine	8 (13.3)
• Body Fat Percentage, (%)	30.13±7.81
• Body Mass Index, (%)	29.45±27.59
• Weight, (Kg)	70.60±18.44
• GOLD classification, n (%)	
A (MIld)	11 (18.3)
B (Moderate)	22 (36.7)
C (Severe)	6 (10)
D (Very Severe)	21 (35)
• Smoking Status, n (%)	
Ex	42 (70)
Never	10 (16.7)
Current	8 (13.3)
LUNG FUNCTION TESTS	
• Predicted FEV_1, (%)	33.92±8.67
• FVC, (%)	63.08±15.84
• FEV_1, (L)	0.98±0.28
• FVC, (L)	2.22±0.69
• FEV_1/FVC, (%)	45.45±17.47
MUSCULAR AND FUNCTIONAL TESTS	

• Maximum Inspiratory Pressure, (cmH_2O)	65.23±24.65
• Maximum Expiratory Pressure, (cmH_2O)	110.35±40.23
• Quadriceps muscle strength (KgF)	21.87±9.39
• Handgrip (KgF)	37.82±8.76
• 5 repetition sit-to-stand, (seconds)	9.73±4.84
• 1 minute sit-to-stand, (repetitions)	27.24±10.26
PATIENT-REPORTED OUTCOMES	
• Frequency of exacerbations, M, (IQR),	1 (0 to 3)
• Modified British Medical Research Council questionnaire, M, (IQR),	2 (1 to 3)
• Borg Scale, M, (IQR),	
Dyspnoea	1 (0 to 3)
Fatigue	0 (0 to 3)
• Hospital Anxiety and Depression Scale M, (IQR),	
Anxiety Score	7 (5 to 11)
Depression Score	8 (3.25 to 9)
• COPD Assessment Test M, (IQR),	20.50 (13.25 to 25)
• St. George Respiratory Questionnaire	
Symptoms score	53.03±20.24
Activity score	68.92±20.63
Impact score	44.18±19.33
Total Score	53.08±17.67

Results are presented in mean ± standard deviation, unless otherwise stated.

COPD- chronic obstructive pulmonary disease; F- female; M-male; FEV_1- forced expiratory volume-one second; FVC- forced vital capacity; M- median; IQR- inter-quartile range.

4.3. Samples quality

4.3.1. Buccal cell samples from Oropharyngeal Swabs

Material from buccal cells collected with the oropharyngeal swabs revealed low DNA concentrations as well as poor A260/280 and A260/230 ratios. Therefore, DNA extraction was not well succeeded and all results were obtained from saliva samples.

4.3.2. Saliva

Eighty-two saliva samples were obtained from patients with COPD at the north and centre regions of Portugal. Only sixty samples were chosen for genotyping, since DNA from twenty-two samples were unsuccessful extracted due to visible impurities (Figure 7) such as food particles (Figure 7b), a brown coloration possibly associated with tobacco (Figure 7c) and in some cases a red coloration (Figure 7e), representing possible lipstick contamination(70).

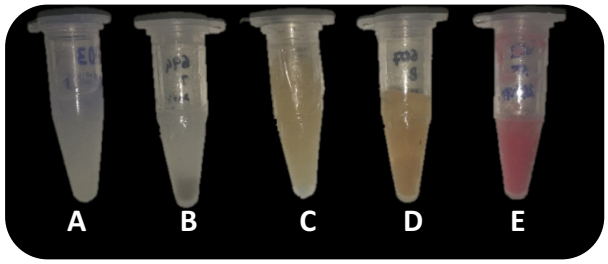


Figure 7- Saliva samples with visible impurities. Sample A-sample with normal appearance; Sample B- agglomerate of food particles and other impurities; Sample C- brown coloration from a smoker patient; Sample D- sample with sputum; Sample E- red coloration from lipstick.

4.4. DNA quantification

DNA was successfully extracted from sixty samples. The yield of DNA obtained was 34,58±30,98 ng/µl measured with Denovix D-11, compared with the 27,71±27,98 ng/µl when measured with Qubit (Table 3). All individual samples measurements (Mean DNA concentrations; A260/280 ratios; A260/230 ratios) assessed with DenoVix D-11 and mean DNA concentration assessed with Qubit may be consulted in Appendix III.

Table 3-Characteristics of the 60 saliva DNA samples that were selected for genotyping analysis. Data for individual samples can be consulted in Appendix III

	Denovix			Qubit
Sample Source	Mean DNA concentration (ng/µl)	Mean±SD A260/280	Mean±SD A260/230	Mean DNA concentration (ng/µl)
Saliva (n=60)	34,58±30,98	1,89±0,10	1,50±0,41	27,71±27,98

Results are mean ± standard deviation

4.5. DNA integrity

Extracted DNA from individual samples were run on agarose (1%) gels. Results of this process revealed that DNA extracted was most of the times intact with minimum degradation as in samples A, C and D. In these samples, a well-defined band was detected (Figure 8). Sample B revealed some degree of degradation (a well-defined band was not observed on the top) however, this was not observed in any other sample.

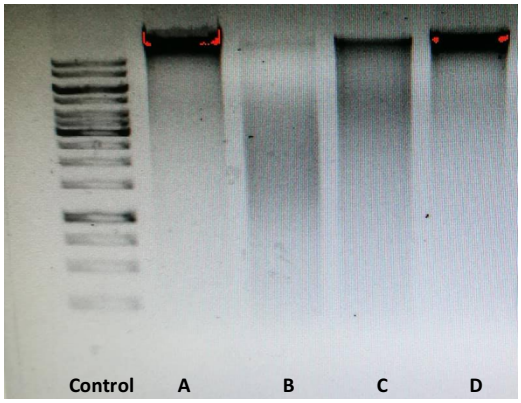


Figure 8 - Agarose gel of four samples to detect degradation.

4.6. DNA samples SpeedVac

DNA samples were concentrated at Speed Vac concentrator to increase the values of DNA concentration required for the genotyping analysis. At the end of the process, the sixty samples obtained at least the minimum values required, reaching a mean DNA concentration of 143.90±110.75 ng/µl and a A260/280 and A260/230 ratios of 1.84±0.09 and 1.27±0.31 respectively (Table 4).

Table 4-Characteristics of the 60 saliva DNA samples selected for genotyping after speed vacuum concentration.

	Denovix		
Sample Source	Mean DNA concentration (ng/µl)	Mean±SD A260/280	Mean±SD A260/230
Saliva (n=60)	143.90±110.75	1.84±0.09	1.27±0.31

Results are mean ± standard deviation

4.7. COPD-related genotyping targets

Results from the systematic review conducted to find genetic variants associated to PROs in COPD, showed that there are twelve SNPs from eight different genes associated with five PROs (Figure 9). Additionally, when a broad COPD-related search was conducted in the literature, more than four hundred SNPs were identified as having significant association with the disease (Appendix II).

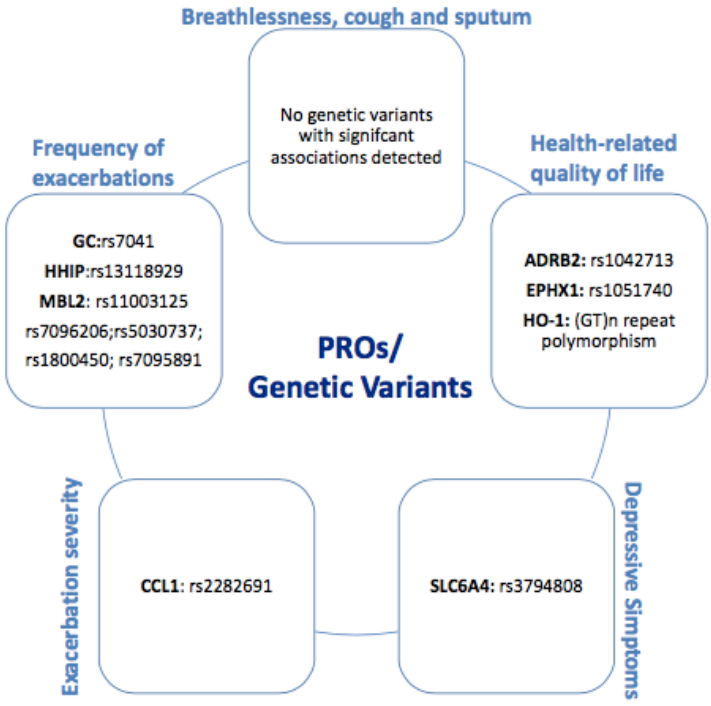


Figure 9- Genetic variants associated with patient-reported outcomes in chronic obstructive pulmonary disease (Adapted from poster (PA4713) at European Respiratory Society (ERS) 2017-Appendix IV).

From this panel of SNPs, one hundred and thirty-five matched those of the Infinium Global Screening Array-24 v1.0 SNPs collection and from the twelve associated with PROs (presented on Figure 9), four matched with the referred array. Samples were genotyped with the GSA microarray under the scope of this dissertation. However, we only had time to analyse the four PRO-related SNPs together with a randomly selected panel of thirty-two SNPs from the one hundred and thirty-five set (rs10011792; rs1042522; rs1042713; rs1042714; rs1042717; rs10461985; rs1051303; rs10516526; rs1051740; rs1059823; rs10844154; rs11001819; rs11046966; rs11172113; rs1129055; rs1130866; rs1131620; rs1138272; rs1143634; rs1155563; rs11556218; rs11614913; rs11677877; rs11739136; rs12477314; rs12504628; rs12899618; rs12922394; rs1303; rs1800450; rs5030737; rs7041) (Figure 10). Further information about the SNPs analysed within the scope of this dissertation may be found in Appendix II.

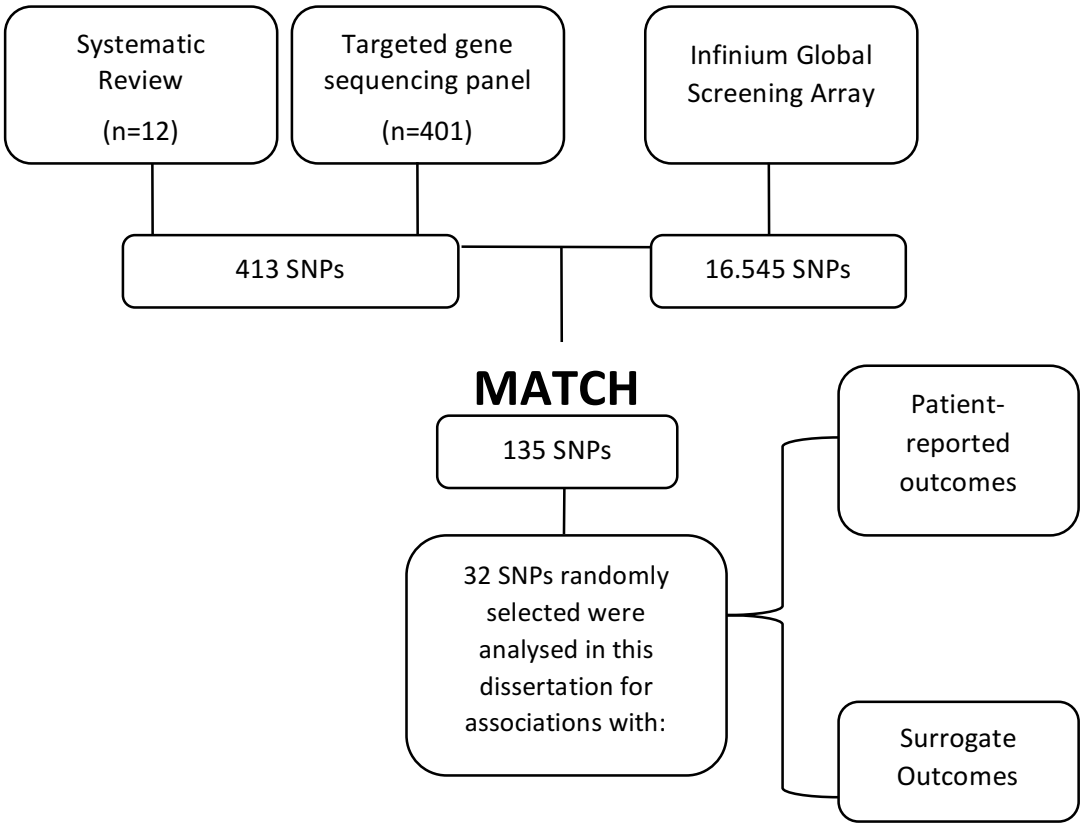


Figure 10-Process conducted to obtain the 32 SNPs analysed in this dissertation.

4.7.1. Significant associations between Patient-reported outcomes and genetic variants

Significant associations were obtained between SNPs and all the PROs assessed, except for self-reported frequency of exacerbations and depression assessed with HADS.

4.7.1.1 Dyspnoea

Significant differences were obtained between genotypes of the SNP rs1143634 and dyspnoea scores assessed with mMRC (*p*=0.009), specifically between the genotypes AA, associated with the highest and GG, associated with the lowest dyspnoea scores (AA: 3 [3 to 3.5] and GG: 2 [1 to 3] *p*=0.012) (Figure 11).

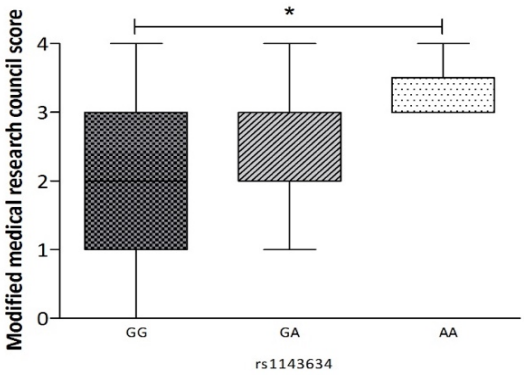


Figure 11-Significant differences obtained between GG and AA genotypes from rs1143634 SNP and dyspnoea scores registered with Modified medical research council. Horizontal lines in the box plot represents the medians, the limits of the box plot represent the 25th and 75th percentiles and the vertical lines (whiskers) are the minimum and maximum values. *p<0.05 (Mann-Whitney U test with Bonferroni correction).

4.7.1.2. Dyspnoea and fatigue

The three SNPs rs1042717, rs1138272 and rs12505628 SNPs were associated with dyspnoea scores assessed with the Borg scale (*p*=0.004, p=0.026 and *p*=0.011 respectively) (Figure 12). In the case of the first SNP (rs1042717), significant differences between the heterozygotic patients, that presented higher values of dyspnoea and the other two genotypes were observed (CC: 0 [0 to 3] and CT: 3 [0.25 to 4], p=0.08; CT: 3 [0.25 to 4] and TT: 0 [0 to 0], *p*=0.013). For the second SNP (rs1138272), significant differences of scores between patients carrying the CC and CT genotypes (CC: 0 [0 to 3] and CT: 3 [2.5 to 4], *p*=0.026) were observed, however patients with the TT genotype were not found in this population. Finally, significant differences of scores were observed between patients with the TT genotype from rs12505628 SNP, who had lower scores of

dyspnoea and patients with TC genotype, who obtained the higher scores (TT: 0 [0 to 1.50] and TC: 3 [0 to 4], *p*=0.004).

Borg scale was also used to assess fatigue (Figure 13). Scores between the rs1042714 genotypes (*p*=0.034) were significantly different, more specifically between patients with the GG and GC genotypes (GG: 0 [0 to 1.25] and GC: 3 [0 to 4] p=0.011). rs1138272 also originated significantly different scores between genotypes, specifically between patients with CC and CT genotypes (CC: 0 [0 to 3] and CT: 3 [1.75 to 4], *p*=0.020). For this last SNP, patients carrying the TT genotype were not found.

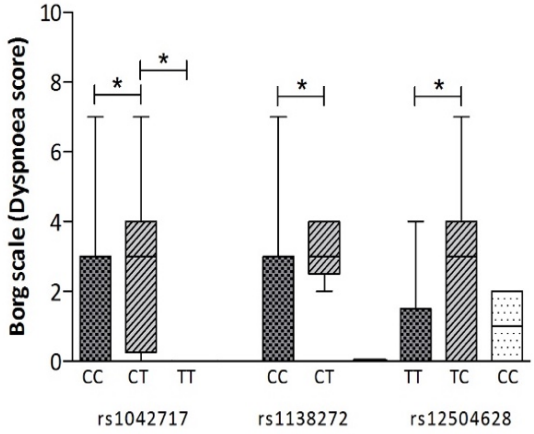


Figure 12-Significant differences obtained between genotypes of rs1042717, rs1138272 and rs12504628 SNPs and dyspnoea scores registered with Borg scale. Horizontal lines in the box plot represent the medians, the limits of the box plot represent the 25th and 75th percentiles and the vertical lines (whiskers) are the minimum and maximum values. *p<0.05 (Mann-Whitney U test with Bonferroni correction).

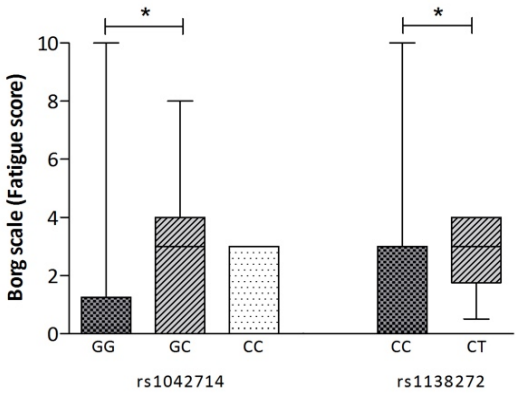
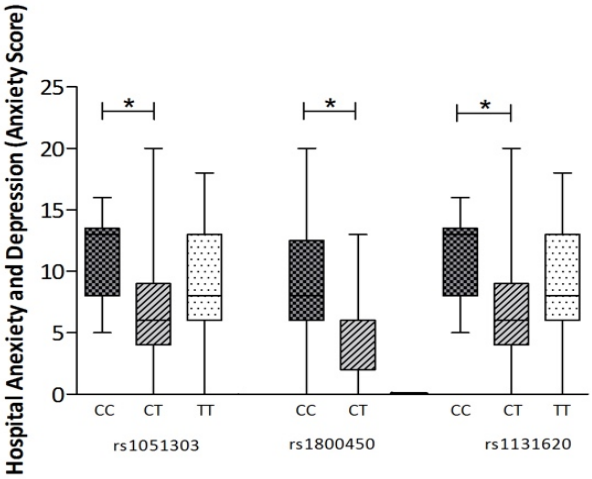


Figure 13-Significant differences obtained between genotypes of rs1042714 and rs1138272 SNPs and fatigue scores registered with Borg scale. Horizontal lines in the box plot represents the medians, the limits of the box plot represent the 25th and 75th percentiles and the vertical lines (whiskers) are the minimum and maximum values. *p<0.05 (Mann-Whitney U test with Bonferroni correction).

4.7.1.3 Anxiety and depression

No SNPs were found associated with depression assessed with HADS, but three significant associations were found between rs1051303, rs1800450 and rs1131620 and anxiety assessed with the same scale (*p*=0.045, *p*=0.017 and *p*=0.024, respectively) (Figure 14). For the first SNP (rs1051303), significant differences of scores between patients carrying the CC genotypes (with higher scores of anxiety) and the heterozygote genotypes (with the lower scores) (CC: 11 [7.50 to 13.75] and CT: 6 [4 to 9.25], *p*=0.016) were observed. Likewise, for the second SNP (rs1800450)

significant differences between the CC and CT genotypes (CC:8 [6 to 12.50] and CT:6 [2 to 6.50], *p*=0.009) were observed. For this last case, patients carrying the TT genotype were not found. Finally, for the rs1131620 SNP, significant differences were observed between patients with CC genotype, with higher scores of anxiety and patients with CT genotype (CC: 13 [8 to 13.50] and CT: 6 [4 to 9.25], p=0.006).



***F*igure 14**-Significant differences obtained between genotypes from rs1051303, rs1800450 and rs1131620 SNPs and anxiety scores registered with Hospital Anxiety and Depression scale. Horizontal lines in the box plot represents the medians, the limits of the box plot represent the 25th and 75th percentiles and the vertical lines (whiskers) are the minimum and maximum values. *p<0.05 (Mann-Whitney U test with Bonferroni correction).

4.7.1.4 Impact of COPD

Impact of the disease was assessed with CAT. rs10461985 and rs11172113 SNPs were significantly associated with CAT scores (*p*=0.048 and *p*=0.008 respectively) (figure 15). For the first SNP (rs10461985), significant differences of scores between patients carrying the GG genotype with higher impact of disease and patients with GA genotype with the lower values (GG: 21 [14 to 26] and GA 12 [7.50 to 16.50], *p*=0.048) were observed. Patients carrying the AA genotype in this population were not found. For the second and last SNP (rs11172113), significant differences of score between patients with AA genotypes, with higher scores and patients with AG genotypes (AA: 23 [20.50 to 28.50] and AG: 15 [8.75 to 21.25], *p*=0.004) were obtained.

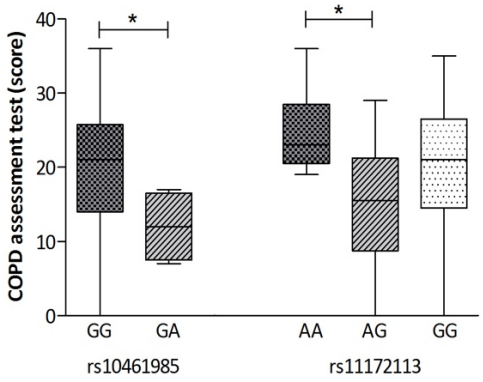


Figure 15-Significant differences obtained between genotypes from rs10461985 and rs1172113 SNPs and health-related quality of life scores registered with Chronic Obstructive Pulmonary Disease Assessment Test. Horizontal lines in the box plot represents the medians, the limits of the box plot represent the 25th and 75th percentiles and the vertical lines (whiskers) are the minimum and maximum values. *p<0.05 (Mann-Whitney U test with Bonferroni correction).

4.7.1.5 Health-related quality of life

HRQOL was assessed with SGRQ and significant associations were found with four different SNPs and the four categories of SGRQ – symptoms, activities, impact of the disease and total score. Symptoms score was significant associated with the rs11172113 SNP (p=0.004) (Figure 16). In this case, significant differences between patients homozygotes AA, with higher score and consequently more severe symptoms and the heterozygotes (AA: 66.93±18.40; AG:42.58±19.78, p=0.006) were found. In the activity score, three significant associations between this outcome and rs1042713, rs11172113 and rs1138272 (p=0.036; p=0.021 and p=0.017 respectively) were found (Figure 17). For the first SNP (rs1042713), significant differences of score between patients carrying the CC genotype, with a higher activity limitation, and patients with the heterozygote genotype (CC:79.03±15.91 and CT:63.23±22.08, p=0.042) were observed. In the second SNP (rs11172113), significant differences between patients carrying the heterozygote genotype and patients carrying the GG genotype (AG:58.79±22.24 and GG:73.90±17.69, p=0.032) were observed. For the last SNP(rs1138272), the heterozygotes CT revealed significant higher values of activity score, comparing to the CC genotypes (CC:66.32±20.24; CT:89.24±13.98, p=0.017). No patients with TT genotype for this last SNP were found. The impact score was associated with two SNPs: rs1138272 (p=0.045) and rs12504628 (p=0.011) (Figure 18). For the rs1138272 SNP case, significant differences between patients carrying the CT genotype, associated with higher impact of the disease, comparing with the CC genotypes (CC: 42.27±18.97 and CT: 60.65±20.15, p=0.045) were observed.

For the rs12504628 SNP, significant differences of scores between patients carrying TT genotype and patients carrying the TC genotype (TT:37.08±16.61; TC=50.27±18.72, *p*=0.032) were observed. Finally, in the total score, similar associations with SNPs previously reported in other categories of this scale were found (rs11172113, *p*=0.006 and rs1138272, *p*=0.021) (Figure 19). For the first SNP (rs11172113), significant differences of score were observed between the patients carrying the heterozygote genotype and patients carrying the AA genotype (AA: 62.12±14.39 and AG 43.53±18.33, *p*=0.020) and between patients carrying the heterozygote genotype and the patients with the GG genotype (AG 43.53±18.33 and GG= 57.00±15.47, *p*=0.021). Finally, for the second SNP (rs1138272), significant differences between patients with the heterozygotes genotypes, with higher score and the CC genotypes (CC: 51.14±17.46 and CT: 70.39±13.48, *p*=0.021) were observed. Patients with the TT genotype were not found for this last SNP.

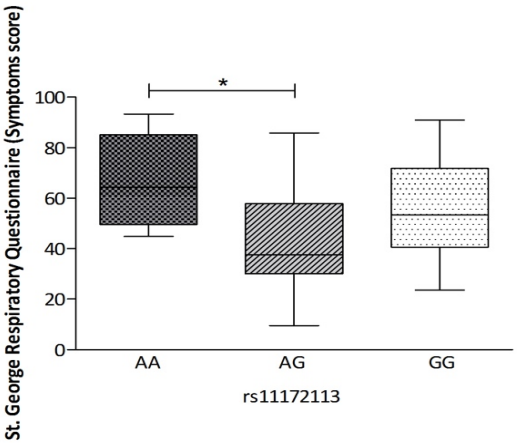


Figure 16- Significant differences obtained between AA and AG genotypes from rs11172113 SNP and health-related quality of life assessed with St. George Respiratory Questionnaire (symptoms score). Horizontal lines in the box plot represents the medians, the limits of the box plot represent the 25th and 75th percentiles and the vertical lines (whiskers) are the minimum and maximum values. *p<0.05 (One-way ANOVA test with Bonferroni correction).

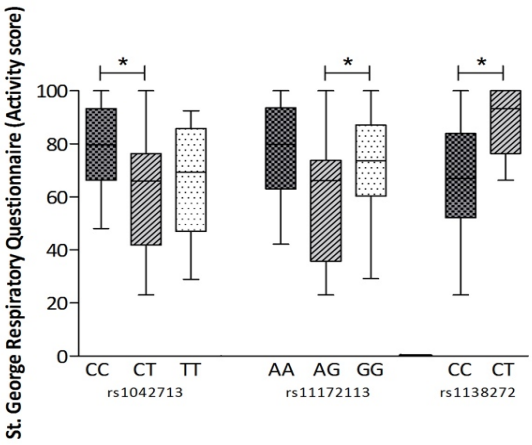


Figure 17- Significant differences obtained between genotypes from rs1042713, rs11172113 and rs1138272 SNPs and health-related quality of life assessed with St. George Respiratory Questionnaire (activity score). Horizontal lines in the box plot represents the medians, the limits of the box plot represent the 25th and 75th percentiles and the vertical lines (whiskers) are the minimum and maximum values. *p<0.05 (One-way ANOVA test with Bonferroni correction).

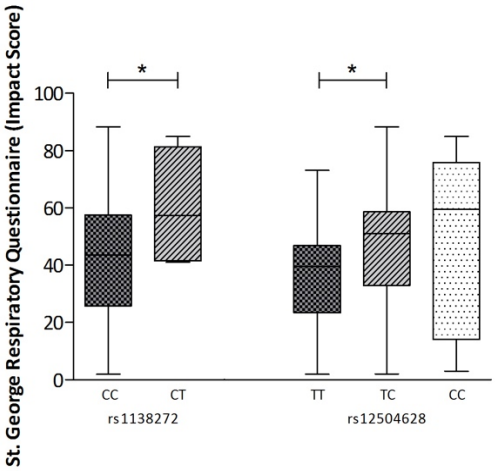


Figure 18- Significant differences obtained between genotypes and rs1138272 and rs12504628 SNPs and health-related quality of life assessed with St. George Respiratory questionnaire (impact score). Horizontal lines in the box plot represents the medians, the limits of the box plot represent the 25th and 75th percentiles and the vertical lines (whiskers) are the minimum and maximum values. *p<0.05 (One-way ANOVA with Bonferroni correction).

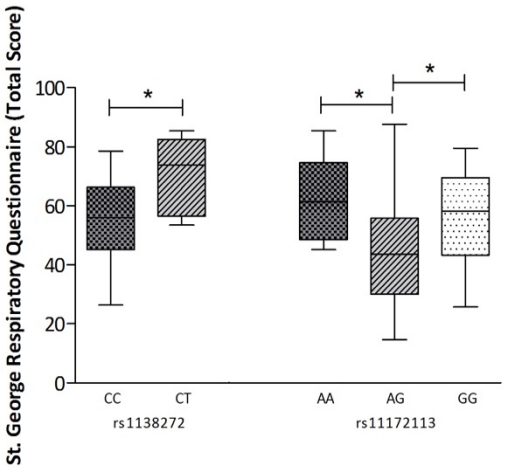


Figure 19- Significant differences obtained between genotypes and rs1138272 and rs11172113 SNP and health-related quality of life assessed with St. George Respiratory questionnaire (total score). Horizontal lines in the box plot represents the medians, the limits of the box plot represent the 25th and 75th percentiles and the vertical lines (whiskers) are the minimum and maximum values. *p<0.05 (One-way ANOVA with Bonferroni correction).

Figure 20 summarizes all the associations found between the SNPs and the PROs in this population.

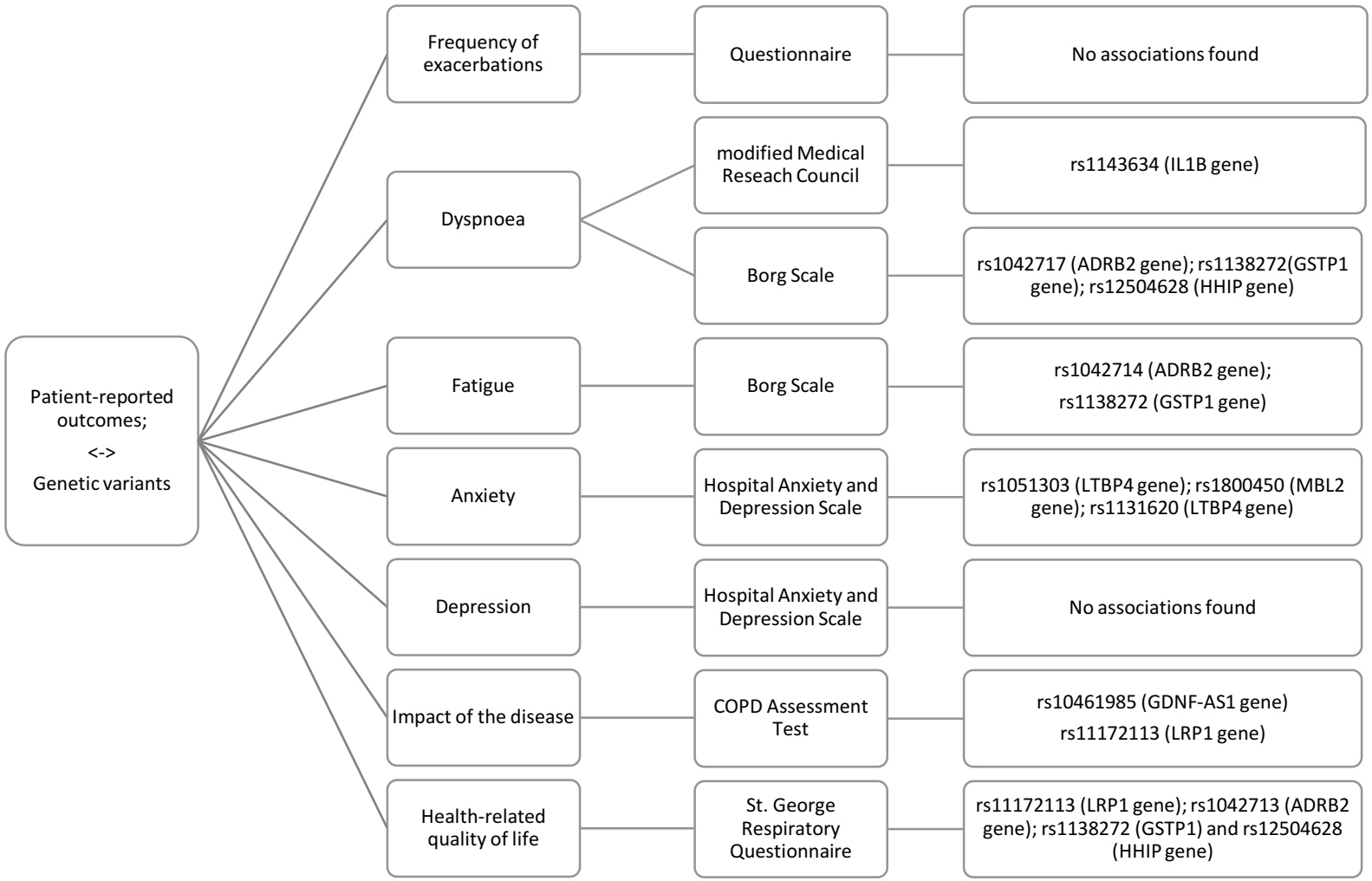


Figure 20-Summary of the associations between single nucleotide polymorphisms, patient-reported outcomes and patient-reported outcomes measures.

4.7.2. Significant associations between Surrogate Outcomes and genetic variants

A total number of nine different SNPs were associated with the surrogate outcomes assessed in patients with COPD. Only maximum inspiratory pressure did not obtain any association.

4.7.2.1 Lung function

A total of four SNPs presented significant associations with FEV_1pp, FVCpp and FEV_1/FVC ratio. Rs1042713 was the SNP significantly associated with FEV_1 pp (*p*=0.027). Significant differences between patients carrying the CC genotype, who had the lower values of FEV_1pp comparing with patients carrying the CT genotype (CC:29.82±8.97%; TC:36.46±7.81%, *p*=0.027) were observed (Figure 21). Additionally, it was also observed that rs1042717 and rs5030737 SNPs had significant associations with FVCpp (*p*=0.039 and *p*=0.007, respectively) (Figure 22). In both cases, significant differences were observed between the heterozygotes, which obtained significant lower values of FVCpp, when compared with patients carrying CC genotype for the rs1042717 SNP (CC:67.27±12.99% and CT:57±17.65%, *p*=0.045) and GG genotype for the rs5030737 SNP (GG:65.20±14.89% and GA:49.33±15.82%, p=0.007). A significant association between this last SNP (rs5030737) and FEV_1/FVC ratio was also found (*p*=0.000) (Figure 23). Patients with heterozygotes genotypes obtained higher ratios when compared with patients with the GG genotype (GG:42.27±13.32% and GA: 66.13±26.74%, *p*=0.000). For this SNP, no patients carrying the AA genotype were found.

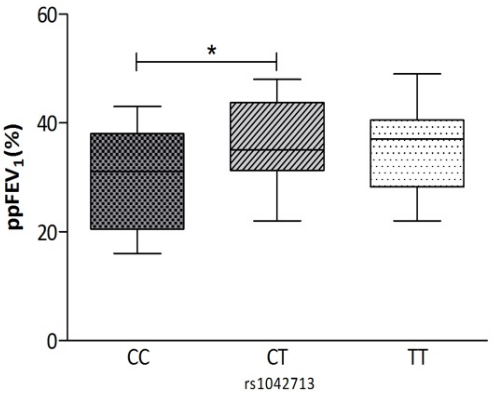


Figure 21-Significant differences obtained between CC and CT genotypes from rs1042713 SNP and FEV1pp. Horizontal lines in the box plot represents the medians, the limits of the box plot represent the 25th and 75th percentiles and the vertical lines (whiskers) are the minimum and maximum values. *p<0.05 (One-way ANOVA with Bonferroni correction).

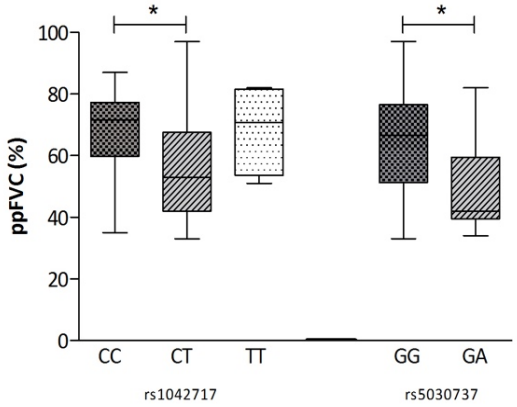


Figure 22-Significant differences obtained between genotypes from rs1042717 and rs5030737 SNPs and FVCpp. Horizontal lines in the box plot represents the medians, the limits of the box plot represent the 25th and 75th percentiles and the vertical lines (whiskers) are the minimum and maximum values. *p<0.05 (One-way ANOVA with Bonferroni correction).

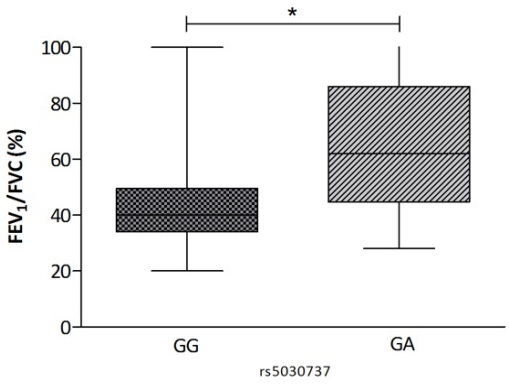


Figure 23-Significant differences obtained between GG and GA genotypes from rs5030737 SNP and FEV1/FVC ratio. Horizontal lines in the box plot represents the medians, the limits of the box plot represent the 25th and 75th percentiles and the vertical lines (whiskers) are the minimum and maximum values. *p<0.05 (One-way ANOVA with Bonferroni correction).

4.7.2.2 Respiratory muscle strength

For respiratory muscles assessment, it was observed that rs1130866 was significantly associated with MEP (p=0.036) (Figure 24). More specifically, significant differences were observed between patients carrying the CC genotype, with higher values of the expiratory muscle strength, compared with patients who had genotype TC (TC:104.67±41.19 cmH_2O; CC: 141.67±32.84 cmH_2O, p=0.03).

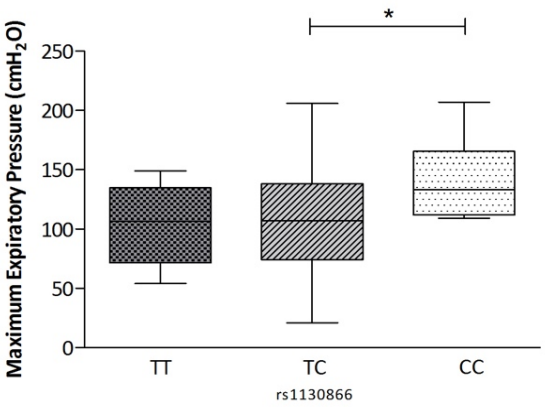


Figure 24-Significant differences obtained between TC and CC genotypes from rs1130666 SNP and maximum expiratory pressure. Horizontal lines in the box plot represents the medians, the limits of the box plot represent the 25th and 75th percentiles and the vertical lines (whiskers) are the minimum and maximum values. *p<0.05 (One-way ANOVA with Bonferroni correction).

4.7.2.3 Peripheral muscle strength

Significant associations between the SNPs rs1042713 and rs1042717 and the quadriceps muscle strength (p=0.043 and p=0.017, respectively) were obtained (Figure 25). In the first case(rs1042713), significant differences of muscle strength were observed between patients carrying the genotype CT, with more quadriceps strength compared with the CC group who were significantly weaker (CC:17.88±9.44 KgF; CT:25.07±9.76 KgF, p=0.038). Furthermore, rs1042717 SNP revealed significant differences in patients carrying the CC genotype that showed more quadriceps muscle strength when compared with the heterozygote genotype (CC:25.20±9.97 KgF; CT:18.33±7.57 KgF, p=0.020).

Upper limb muscle strength also originated significant differences between other two SNPs (rs11172113, p=0.011 and rs11556218, p=0.021) (Figure 26). In the case of the first SNP(rs1172113) a significant difference between the heterozygotes which presented higher values of upper limb muscle strength and patients with the genotype GG who showed the lowest values (AG=42.35±7.23

KgF and GG= 33.56±7.00 KgF, *p*=0.009), was observed. Additionally, for the second SNP (rs11556218), patients carrying the TT genotype were significantly weaker compared to the patients carrying the heterozygote genotype (TT= 36.05±8.14 KgF; TG=43.30±8.74 KgF, p=0.021). In this population, patients carrying the genotype GG were not found.

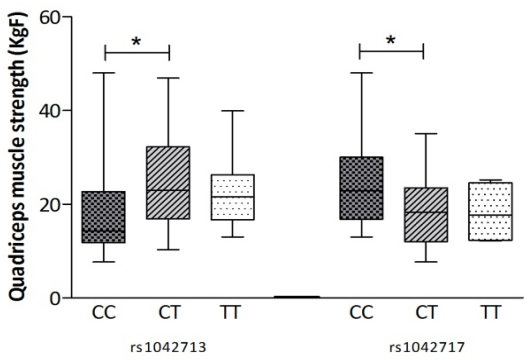


Figure 25-Significant differences obtained between genotypes from rs1042713 and rs1042717 SNP and quadriceps muscle strength assessed with dynamometer. Horizontal lines in the box plot represents the medians, the limits of the box plot represent the 25th and 75th percentiles and the vertical lines (whiskers) are the minimum and maximum values. *p<0.05 (One-way ANOVA with Bonferroni correction).

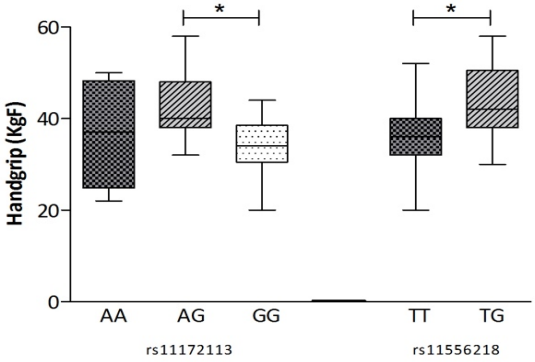


Figure 26- Significant differences obtained between genotypes from rs11172113 and rs11556218 SNP and handgrip test. Horizontal lines in the box plot represents the medians, the limits of the box plot represent the 25th and 75th percentiles and the vertical lines (whiskers) are the minimum and maximum values. *p<0.05 (One-way ANOVA with Bonferroni correction).

4.7.2.4 Functional capacity

Finally, for the functional capacity, significant associations were also obtained with three distinct SNPs and the 5STS and 1 minute STS tests. Rs12899618 revealed associations with the 5STS test (*p*=0.018) (Figure 27). For this SNP, it was possible to observe significant differences between patients with the heterozygote genotype, slower to conduct the five repetitions, compared with patients with the genotype CC (CC: 8.84±3.26 seconds; CT: 12.45±7.51 seconds, *p*=0.018). For this SNP, patients with the genotype TT were not found. 1 minute STS also found significant associations with the two SNPs rs11046966 (p=0.013) and rs1138272 (*p*=0.012) (Figure 28). For the first SNP(rs11046966), significant differences were observed between patients carrying the genotype GG, which performed a higher number of repetitions compared with patients with the AA genotype (AA: 24 [18 to 29] repetitions and GG: 35.50 [29 to 47] repetitions, *p*=0.005). In the second SNP (rs1138272), patients with the CC genotype conducted more repetitions that patients with CT

genotype (CC: 29 [22.75 to 34.25] repetitions and CT: 20 [14 to 21.50] repetitions, *p*=0.012). For this last SNP, there were no patients carrying the TT genotype.

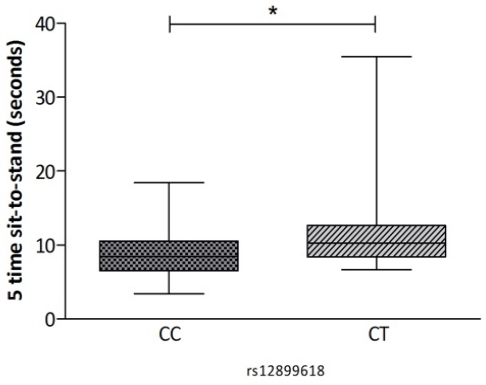


Figure 27-Significant differences obtained between genotypes from rs12899618 SNP and 5 time sit-to-stand test scores. Horizontal lines on the box plot represents the medians, the limits of the box plot represent the 25th and 75th percentiles and the vertical lines (whiskers) are the minimum and maximum values. *p<0.05 (One-way ANOVA with Bonferroni correction).

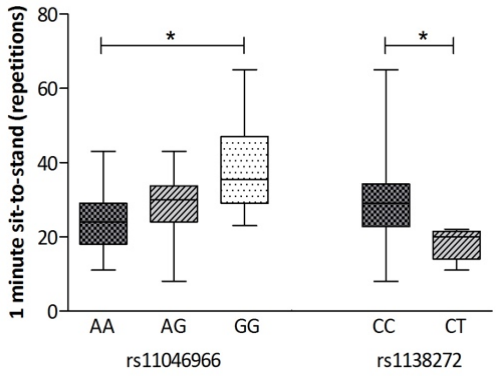


Figure 28-Significant differences obtained between genotypes from rs11046966 and rs1138272 SNPs and 1 minute sit-to-stand test scores. Horizontal lines on the box plot represents the medians, the limits of the box plot represent the 25th and 75th percentiles and the vertical lines (whiskers) are the minimum and maximum values. *p<0.05 (One-way ANOVA with Bonferroni correction).

Figure 29 summarizes all the associations found between the SNPs and the surrogate outcomes assessed.

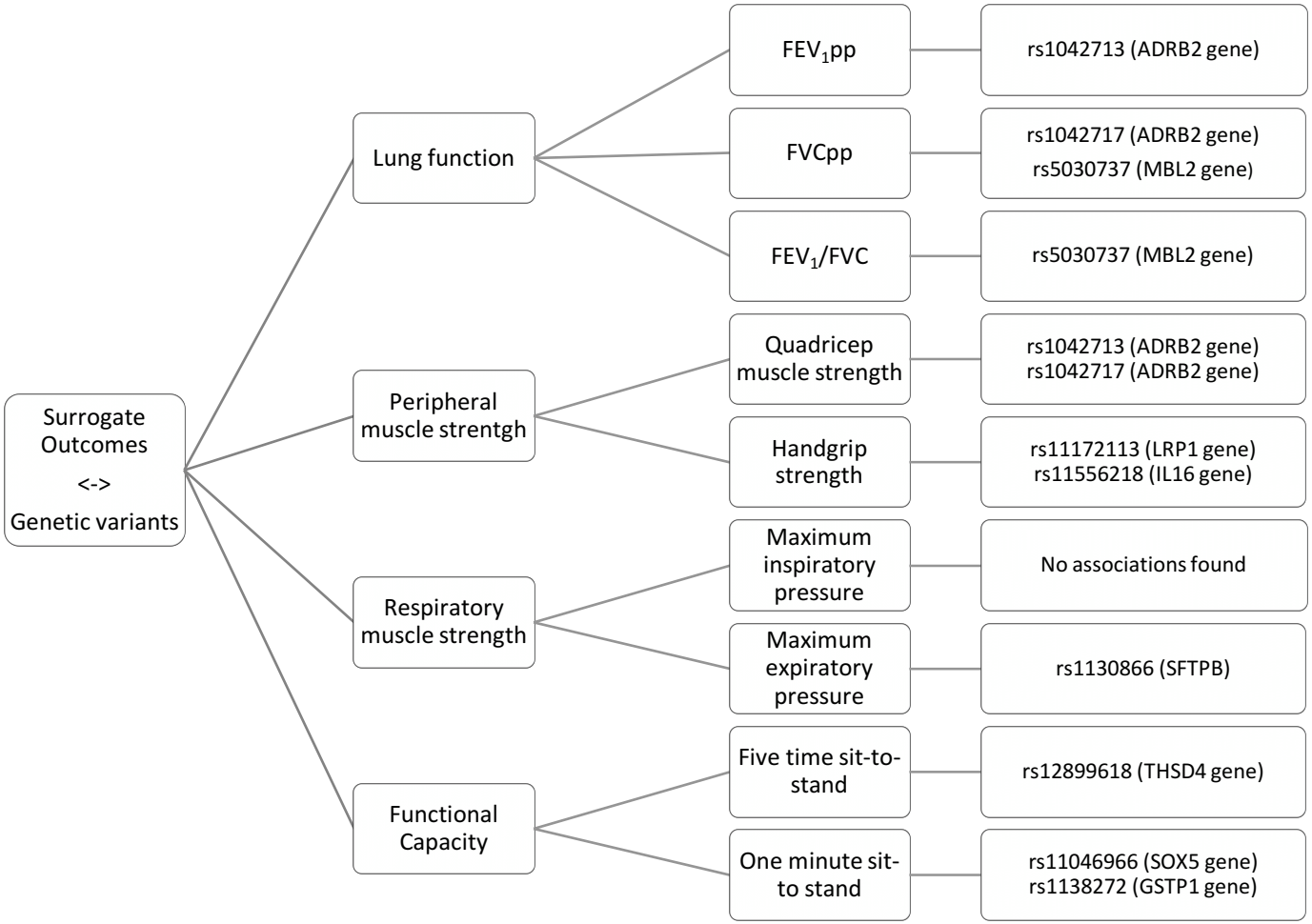


Figure 29- Summary of the associations between single nucleotide polymorphisms and surrogate outcomes and outcome measures.

5. DISCUSSION

5.1. Overview

The first objective of this dissertation was to conduct a customized genotyping for patients with COPD using the SNPs from the targeted gene sequencing panel. Due to economic and technical reasons, this study opted for a genotyping array non-directed for these SNPs, but commonly used to stratify human populations (GSA array). Thus, we were limited to the intersection between our SNPs of interest and the list of SNPs which composed the array. This intersection, by one hand limited our analysis to only 135 SNPs previously associated with COPD in the literature, 32 of which were analysed in this dissertation. On the other hand, this chip revealed genotypes of our patients in relation to thousands of SNPs, some of which have never been studied for this disease, providing the opportunity of carrying out new studies.

5.2. Samples quality and quantity

Our first idea was to obtain DNA from cells using oropharyngeal swabs. This method is non-invasive, usually fast and very easy to apply. The protocol of sample collection had a minimum duration of an hour and half and was often performed in hospital contexts, thus, swabs seemed to be the best way to obtain genetic data in a quick and efficient way. However, this was not verified. We had several difficulties during the DNA extraction, because only residual and insufficient amounts of DNA were obtained. The poor yields may be due to degradation of DNA from buccal cells which may influence the genotyping efficiency(71). One of the options to solve this problem was to increase the number of swabs collected by patient, which would be economically unbearable, would cause patients' discomfort and would cause DNA extraction process more timing-consuming. Other option was to change the source of DNA. According to Hansen et al(72), the use of saliva is the best alternative to obtain DNA with good quality. Thus, saliva samples were obtained from all patients under study. Nevertheless, impurities and an excess of sputum were detected in a few samples. These impurities, in some cases, led to QIAamp Mini spin column obstruction. To overcome this, we increased the time of vortexing, the number of washing steps and incubation time before elution. Despite the difficulties of COPD patients to produce saliva due to the effects of bronchodilator(73), with few millilitres (1-2) we obtained enough DNA to perform genotyping analysis.

5.3. Microarray genotyping

5.3.1. Associations between Patient-reported Outcomes and genetic variants

A total number of eleven different SNPs from 8 genes were associated with all the PROS assessed, except for the frequency of exacerbations and depression score assessed with HADS.

According to the systematic review performed (Appendix I), it was expected to find associations between rs7041, rs5030737 or rs1800450 SNPs from *MBL2* gene and frequency of exacerbations, but this has not occurred. Possible explanations for this absent association are the different characteristics between this population and the populations of other studies, different environments or different exposure to risk factors.

Dyspnoea was assessed using two different scales, mMRC and Borg. Both obtained significant associations with SNPs, but none of the SNPs was common to both scales. Although dyspnoea is a symptom with a single interpretation, both scales assessed it differently. mMRC scale assesses the dyspnoea associated with activities of daily life whereas Borg scale assesses dyspnoea at the exact moment of the evaluation(74). For this reason, patient's response to both cases could have been different, which may explain our result. Homozygotes AA for the SNP rs1143634 obtained the highest values of dyspnoea assessed with mMRC. Based on these results, we can assume that the allele A was the risk allele for dyspnoea. However, this is in contrast with some results of the literature. Some studies have reported that this SNP does not have any association with COPD(75) and others have found the A allele of this SNP with a protective role against lung cancer in COPD(76). Therefore, as in our study, this allele does not seem to be protective in all outcomes of the disease. Furthermore, the associations of rs1042717 SNP from *ADRB2* gene with dyspnoea assessed with the Borg scale was an expected result. *ADRB2* encodes a receptor involved on the relaxation and dilatation of smooth muscles(77). In addition, it has been already reported that the rs1042717 SNP is significantly associated with airway wall thickness(78) and bronchodilator response(79), so its association with dyspnoea, could have been expected.

Anxiety score obtained with HADS was significantly associated with three SNPs, two of which from the *LTBP4* gene (rs1051303 and rs1131620). Both SNPs have been previously reported as being associated with low exercise capacity and 6 minutes walking distance in patients with COPD(80). Although these SNPs were not directly associated with anxious behaviours, it has been previously reported that physical activity deficits may lead to obesity and increase depressive symptoms and anxiety (81). Thus, the association of these SNPs with anxious behaviours seems to

be somewhat expected also in patients with COPD, emphasizing once again the importance of analysing not only genetic variants directly associated with the outcomes, but also other variants indirectly associated, as well as possible interactions between these different genetic variants.

HRQOL and impact of the disease were assessed with two different tools, SGRQ and CAT, respectively. Since these two scales are strongly correlated with each other, and patients that usually presented higher impact of the disease, also had worst HRQOL(82), we expected to find some common SNPs to both scales. Significant associations with five different SNPs were obtained, however, one specific variant (rs11172113 from *LRP1* gene) was common to both scales (CAT score, and SGRQ symptoms, activity and total scores). This SNP was previously associated to lung function in the literature, where patients carrying the risk allele A had lower FEV_1 and FEV_1/FVC values(83). Therefore, since patients with airflow limitations usually show higher impact of the disease(84) and worst HRQOL(85), it was expected that patients carrying genotypes with this allele had higher impact of the disease and consequently worst HRQOL. In this study, patients carrying the AA genotype reported higher scores in CAT, which represents a higher impact on patient life's. A similar effect was observed on symptoms and total score obtained with SGRQ, where homozygotes patients AA, once again, obtained higher scores. Surprisingly, on the SGRQ activity domain, patients with the GG genotype obtained higher scores, very similar to those patient's homozygotes to the risk allele (AA). This was not expected, since this genotype does not have the risk allele, and this may be due once again to the small sample size. Finally, it is important to point out that in all cases (CAT and SGRQ), patients carrying the heterozygote genotype for this SNP always obtained the best scores for disease impact and HRQOL. Thus, despite the fact that patients with this genotype also had a risk allele, we suspect that this genotype may confer protection to patients with COPD at least in this population.

5.3.2. Associations between Surrogate Outcomes and genetic variants

Significant associations were observed between nine different SNPs and all clinical outcomes assessed except for MIP.

Two different SNPs affecting ADRB2 (rs1042713 and rs1042717) and MBL2 (rs5030737) genes were significantly associated with lung function. It was expected that some SNPs of the ADRB2 gene were associated with this outcome, for the same reason that were previously associated to dyspnea. Patients with TT genotype (rs1042713), for example, were previously associated with better improvements in spirometric measures and HRQOL when treated with anticholinergic bronchodilators(86). However, in this population, patients with the heterozygote

genotype (TC) revealed higher values of FEV_1pp, but with values very similar with previously referred TT genotype (TC: 36.46±7.81% vs TT:34.90±7.73%). Additionally, we did not expect to find SNPs of MBL2 (rs5030737) associated with lung function. This SNP, as observed in our systematic review, was associated with the frequency of exacerbations in patients with COPD, where patients containing genotypes with allele A were more susceptible to have higher frequency of exacerbations(87). Thus, patients carrying this risk allele may have a worst lung function, possibly due to the damage caused by the high number of exacerbations(88). For this SNP, patients carrying the GA genotype had lower values of FVCpp, and consequently also had higher values of FEV_1/FVC ratio, as expected.

From all variants associated with muscle strength in this study, none was directly associated with this phenotype in patients with COPD in the literature, up to this point. In fact, de vast majority of these SNPs have been previously associated to lung function (89-91). Nevertheless, previous studies already reported significant correlations between lung function (FEV_1) and muscle endurance(92) and lung function and quadriceps muscle strength(93), where patients with lower FEV_1 also had weaker quadriceps muscle strength and less muscle endurance. Thus, it seems likely that SNPs with genotypes responsible for lower FEV_1 may also influence negatively the muscle strength of the patient. For example, relative to the rs1042713 SNP affecting the ADBR2 gene, significant associations were found in both lung function and quadriceps muscle strength, assessed with hand-held dynamometer. Patients with CC genotypes were associated to lower values of FEV_1pp (lung function) and also to the lowest values of quadriceps strength. However, this was not verified in all of cases. Rs1130866 SNP (*SFTPB* gene) was, in this study, significantly associated with respiratory muscle strength as assessed through MEP. Previous reports showed that patients with TT genotype had higher values of FEV_1pp(90), thus following the same rationale, it was expected that patients with the same genotype would also show higher values of MEP. However, in this specific case, patients carrying the TT genotype showed the lowest values of MEP, contrary to what we expected. This difference of what was expected and what was observed may be due to the fact that only a single SNP may not explain this dysfunction on the respiratory muscles. Despite this, interactions between genes or an interaction between this gene and the environment may lead to distinctive values. For example, the common practice of physical activity or integration in a rehabilitation program, may lead to values completely different of what was expected.

5.3.3. Limitations

This work had some limitations that need to be considered. The reduced sample size, may had some influence in our results, for example it may explain why some of genotypes of the SNPs were not found. Furthermore, there were also some missing clinical data which could be important and may have influenced our results. Plus, we had not a control population and we did not take into account some variants as gender, weight, body mass index.

6. CONCLUSIONS AND FUTURE RESEARCH

In this dissertation, we assessed which specific genetic variants may be associated with PROS and surrogate outcomes commonly used in patients with COPD. To our best knowledge, this was the first study reporting associations between SNPs and some of the PROs (i.e., CAT, mMRC and Borg) and clinical variants (i.e., MEP, handgrip, 5STS and 1 minute STS) in patients with COPD. Significant associations were observed between all the PROs and surrogate outcomes, except for self-reported frequency of exacerbations, depression and MIP. This allow us to conclude that genetics may play a very strong role not only in the susceptibility to develop COPD, but also in almost all outcomes of this disease. Furthermore, our results also showed new associations between some of the genetic variants and outcomes which were not directly connected. This reinforces what other studies had already observed: COPD is a complex and heterogeneous disease that cannot be explained by a single SNP, thus, it is important to also understand the gene-gene and gene-environment interactions(91, 94)

In future studies, the sample size will be increased and an age/gender matched control population will be added to assess whether significant associations obtained in this study will be maintained. We also intend to assess the effect of other variables, such as, the stage of the disease, gender, smoking status and BMI, on the obtained results. The remaining SNPs genotyped for this population and the other SNPs of the chip will also be analysed and new PROs and surrogate outcomes will be included.

Our investigation opened paths to the knowledge of the genetic variants associated with the different outcomes of COPD and unravel the possibility, in a near future, to anticipate patients' behaviour and adjust tailored treatments.

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Annex I – Ethics' approval

GOVERNO DE PORTUGAL
MINISTÉRIO DA SAÚDE

ARSC ADMINISTRAÇÃO REGIONAL DE SAÚDE DO CENTRO, I.P.

COMISSÃO DE ÉTICA PARA A SAÚDE

PARECER FINAL:	DESPACHO:
Parecer **favorável**	Deliberado Homologar o parecer favorável 16.11.03 Conselho Directivo da A.R.S. do Centro, I.P. Dr. José Manuel Azenha Tereso Presidente, Dr. Luís Manuel Militão Mendes Cabral Vogal, Dr. Mário Ruivo Vogal,

ASSUNTO: **64/2016 - "GENIAL – Marcadores genéticos e clínicos na trajetória da DPOC"**

Este projeto, financiado pela Fundação para a Ciência e Tecnologia ref. PTDC/DTPPIC/2284/2014, visa determinar o papel das mutações genéticas associados ao desenvolvimento e trajetória da DPOC e identificar os marcadores clínicos (e.g., dispneia; número de exacerbações; função pulmonar; tolerância ao exercício) capazes de detetar episódios de EADPOC. A Doença Pulmonar Obstrutiva Crónica (DPOC) é progressiva e caracteriza-se por exacerbações agudas (EADPOC) frequentes. Devido à sua elevada morbilidade, mortalidade e custos associados, a DPOC constitui um grave problema de saúde pública. A prevenção, diagnóstico e monitorização da DPOC são, por isso, prioridades para os sistemas de saúde mundiais e tópicos atuais de investigação. Pouco se sabe também acerca da relação entre o curso individual da DPOC e o perfil genético dos pacientes. A identificação e tratamento precoce das EADPOC têm mostrado reduzir os internamentos hospitalares e o tempo de recuperação dos pacientes, e estão associados com uma progressão mais lenta da doença e uma redução na sobrecarga total e custos diretos atribuíveis à DPOC.3, 10, 11 Assim, é essencial identificar os marcadores clínicos que são sensíveis a alterações no estado de saúde dos pacientes ao longo do tempo e podem detetar EADPOC numa fase inicial.

Isto irá permitir o desenvolvimento de um algoritmo clínico para melhorar o diagnostico e intervenção mais eficaz das EADPOC.

O projeto é inovador, está bem explicitado e estruturado. Justifica corretamente a amostra utilizada.

Coimbra, 26 de outubro de 2016

A Relatora
(Dra. Carla Barbosa)

O Presidente da CES
(Prof. Dr. Fontes Ribeiro)

DECLARAÇÂO

Eu, Manuel Duarte Rezende Pereira Sebe, Director Executivo do Agrupamento dos Centros de Saúde do Baixo Vouga, autorizo a realização do estudo "Genial – Marcadores Genéticos e Clínicos na Trajetória da DPOC", da responsabilidade da investigadora principal Dra. Alda Sofia Pires de Dias Marques da Universidade de Aveiro, garantindo as condições necessárias à sua participação.

Aveiro, 08 de Março de 2017

O Director Executivo do ACeS Baixo Vouga

Dr. Manuel Sebe

Manuel Sebe, Dr.
Director Executivo
ACES BAIXO VOUGA

UNIDADE LOCAL DE SAÚDE DE MATOSINHOS HOSPITAL PEDRO HISPANO	INFORMAÇÃO	Nº 10/CE/JAS Data: 17-02-2017

Para: **Serviço de Gestão do Conhecimento**
De: **Comissão de Ética**

Assunto: Pedido de autorização para realização de estudo intitulado "**GENIAL – Marcadores genéticos e clínicos na trajectória da DPOC**"

INFORMAÇÃO

Exmos. Senhores,

A Comissão de Ética analisou o pedido de autorização para realização de estudo intitulado "**GENIAL – Marcadores genéticos e clínicos na trajectória da DPOC**", financiado pela Fundação para a Ciência e Tecnologia, proponente Alda Sofia Pires de Dias Marques, docente e investigadora da Escola Superior de Saúde (ESSUA) e Instituto de Biomedicina (iBIMED) da Universidade de Aveiro.

Decidido nada a opor à realização do estudo.

Com os melhores cumprimentos

Dr.
Presidente
da ULSM

Dr. José Alberto Silva
(Presidente da Comissão de Ética da U. L. S. – Matosinhos)

De: **Alda Marques** amarques@ua.pt
Assunto: FW: Estudo Clínico intitulado "GENIAL - Marcadores genéticos e clínicos na trajectória do DPOC
Data: 18 de julho de 2017, 15:22
Para: Ana Machado filipamachado@ua.pt, Cátia Paixão catia.paixao@ua.pt, Hélder Melro heldermelro@ua.pt, Ana Oliveira alao@ua.pt, Sara Miranda sara.souto@ua.pt

From: hdff [mailto:hdff@hdfigueira.min-saude.pt]
Sent: 18 de julho de 2017 10:59
To: Alda Marques <amarques@ua.pt>
Cc: Maria Manuela Lourenço Lopes <manuelalopes@hdfigueira.min-saude.pt>; Direção Clinica <dir.clinica@hdfigueira.min-saude.pt>
Subject: Estudo Clínico intitulado "GENIAL - Marcadores genéticos e clínicos na trajectória do DPOC

Dra. Alda Pires de Dias Marques,

Encarrega-me o Dr. José Albino e Silva, Presidente do Conselho de Administração de informar que o Conselho de Administração deliberou aprovar o pedido apresentado para a realização do Estudo Clínico intitulado "GENIAL – Marcadores genéticos e clínicos na trajectória do DPOC".

Com os meus cumprimentos,

Ana Maria Rodrigues
Secretariado do Conselho de Administração

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Appendix I: Publication I (under review at PLOS One-PONE-D-17-33441)- Genetic profile and patient-reported outcomes(PROs) in Chronic Obstructive Pulmonary Disease: a systematic review.

Genetic profile and patient-reported outcomes PROs in Chronic Obstructive Pulmonary Disease: a systematic review

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Abstract

Background: Chronic Obstructive Pulmonary Disease (COPD) impacts differently on patients at similar grades, suggesting that factors other than lung function may influence patients' experience of the disease. Recent studies have found associations between genetic variations and patient-reported outcomes (PROs). Identifying these associations might be fundamental to predict the disease progression and develop tailored interventions. This systematic review aimed to identify the genetic variations associated with PROs in COPD.

Methods and Findings: Databases were searched until July 2017 (PROSPERO: CRD42016041639) and additional searches were conducted scanning the reference list of the articles. Two independent reviewers assessed the quality of studies using the Q-Genie checklist. This instrument is composed of 11 questions, each subdivided in 7 options from 1 poor-7 excellent.

Thirteen studies reporting 5 PROs in association with genes were reviewed. Studies were rated between "good quality" (n=8) and "moderate" (n=5). The most reported PRO was frequency of exacerbations (n=7/13), which was mainly associated with MBL2 gene variants. Other PRO's were health-related quality of life (HRQOL) (n=4/13), depressive symptoms (n=1/13), exacerbation severity (n=1/13) and breathlessness, cough and sputum (n=1/13), which were commonly associated with other genetic variants.

Conclusions: Although a limited number of PRO's have been related to genetic variations, findings suggest that there is a significant association between specific gene variants and the number/severity of exacerbations, depressive symptoms and HRQOL. Further research is needed to confirm these findings and assess the genetic influence on other dimensions of patients' lives, since it may enhance our understanding and management of COPD.

Introduction

Chronic obstructive pulmonary disease (COPD) is a multifactorial, heterogeneous and progressive condition that affects 210 million people worldwide(1). Severity of COPD is usually classified according to the degree of airway obstruction (assessed with spirometry), nevertheless it has been acknowledged that people at similar grades of COPD report different disease impacts(1). These different reports among patients suggest that factors beyond lung function influence patients' experience of the disease. Indeed, upstream factors, such as the presence of specific genetic variants, have already been reported to play a role in this matter(2). For example, polymorphisms in SERPINA1 usually lead to a deficiency of the α1 antitrypsin, affecting 1-2% of all COPD cases(3). Additionally, the role of other candidate genes in the pathogenesis, comorbidities and outcomes of the disease have been studied(4).

Patient-reported outcomes (PROs) are a set of health outcomes directly reported by patients and may include symptoms (dyspnea, cough, pain, fatigue), exacerbation frequency and health status, among others (5). These outcomes are accepted as the most faithful representation of patients' perspectives of the impact of the disease and treatment benefits(6). The needed of assessing PROs has been considerably highlighted in the Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2017 update, which suggests that COPD classification should now be based on exacerbation frequency and patient's perception of their symptoms rather than on lung function only (1).

In the last years, it has become more evident that there is a strong association between PROs and genetics, namely in lung cancer(7), which may suggest that strong associations may also exist between genetics and PROs in COPD. However, a review of the known correlations between these two factors in COPD has never been conducted. The combination of genetics and PROs would be valuable to identify patients susceptible to PROs deficits, understand the diagnosis, predict disease progression and develop tailored and timely interventions (8).

Therefore, the focus of this systematic review was to synthetize the genetic variations associated with different PROs in COPD.

Methods

Search strategy

The systematic review protocol was registered at Prospective Register of Systematic Reviews (PROSPERO) (ref CRD42016041639**).** A comprehensive systematic search was conducted in May 2016 and weekly updates were performed until July 2017 in the following medical databases: PubMed (1950-2016), Scopus (1960-2016) and Web of Science (1900-2016). The PICOS (Populations, Intervention, Comparison, Outcome and Study Design) framework was used to develop literature search strategies, however Intervention (I) and Comparison (C) terms were omitted as they were not applicable to the present review (9). Accordingly, the search terms were based on a combination of the following keywords: [(COPD OR "chronic obstructive pulmonary disease" OR emphysema OR "chronic bronchitis") AND ("genetic associations" OR "genetic profile" OR "genetic analysis" OR gene) AND (dyspnea OR dyspnoea OR breathlessness OR fatigue OR cough OR depression OR anxiety OR "daily living" OR "quality of life" OR mood OR "well-being" OR “frequency of exacerbation” OR exacerbations OR "hospital admissions" OR "hospital length of stay" OR "acute exacerbations" OR “physical activity” OR "physical fitness" OR “physical function” OR “sputum production” OR phlegm OR pain OR “patient-reported outcomes” OR “patient-centered outcomes” OR “patient-centered outcomes”)]. The reference lists of the selected articles were also scanned for other potential eligible studies.

Eligibility criteria

Studies were considered eligible if included adult patients (>18 years old) diagnosed with COPD and associated a genetic profile to one or more PRO. For the purpose of this systematic review, PRO

were defined, according to the Cochrane Collaboration definition, as “reports coming directly from patients about how they feel or function in relation to a health condition and its therapy without interpretation by healthcare professionals or anyone else” (10). Studies were excluded if were conducted in animals, were written in languages other than English, Spanish, French or Portuguese and did not differentiate chronic obstructive diseases (i.e., presented pooled data from several chronic obstructive diseases such as asthma, COPD and bronchiectasis). Book chapters, review papers, abstracts of communications on meetings, letters to the editor, commentaries to articles, unpublished work and study protocols were not considered suitable and, therefore, were also excluded from this study. This systematic review was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) (9, 11).

Quality Assessment

The quality, internal validity and risk of bias of the included studies were assessed using the Quality of genetic association studies checklist (Q-Genie) (12). This instrument is composed of 11 questions to assess “rationale for study”, “selection and definition of outcome of interest”, “selection and comparability of comparison groups”, “technical classification of the exposure”, “non-technical classification of the exposure”, “other source of bias”, “sample size and power”, “a priori planning of analysis”, “statistical methods and control for confounding”, “testing of assumptions and inferences for genetic analysis” and “appropriateness of inferences drawn from results”. The Q-Genie checklist has 7 possible answers for each question (i.e., “1 (poor)”, “2”, “3 (good)”, “4”, “5 (very good)”, “6”, “7 (excellent)”). The overall quality of studies is classified as “poor quality” if score is ≤35, “moderate quality” if score is >35 and ≤45, and “good quality” (>45), for studies having control groups. For studies without control groups the values for the parameters listed above are

≤32, >32 and ≤40, and >40, respectively(12). Two reviewers assessed the quality of studies independently. Disagreements were solved consulting a third reviewer.

Studies selection and data extraction

First, duplicates were removed and one reviewer performed the initial screening of title, abstract and keywords of studies based on the type of publication and relevance for the scope of the review. Then the full-text of each potentially relevant study was screened for content to decide its inclusion in the review. For each accepted study, one reviewer extracted the following data to a previously structured table: last author's name and year of publication, study design, sample characteristics (i.e., sample size, age, gender and COPD severity), PRO evaluated and outcome measures used, gene associated with the identified PRO and type of association between the PRO and the identified gene. Two independent reviewers further checked the extracted data for accuracy and completeness. Reviewers resolved disagreements by consensus.

Data analysis

The consistency of the studies quality assessment, performed by the two reviewers, was explored with the Cohen's kappa. The value of Cohen's kappa vary from 0 to 1 and can be interpreted as: i) 0.00-0.20: slight agreement; ii) 0.21-0.40: fair agreement; iii) 0.41-0.60: moderate agreement; iv) 0.61-0.80: substantial agreement; v) 0.81-1.00: almost perfect agreement(13). Statistical analysis was performed using IBM SPSS Statistics version 24.0 (IBM Corporation, Armonk, NY, USA).

Results

Search strategy

The database search identified 1889 studies of potential interest. After duplicates removal, 1259 articles were analyzed for relevant content. From these, 1206 were excluded due to the following reasons: non-original articles (n=575), absence of PRO/genetic associations (n=387), non-specific for COPD (n=122), studies conducted in animals (n=98), studies written in other languages rather than English, Spanish, French or Portuguese (n=24). The full-text of the remaining 53 potentially relevant articles was assessed and 40 articles were excluded. Reasons for exclusion included: absence of PRO (n=19), absence of genetic association (n=19), not specific for COPD (n=1) and unavailability of the article even after contacting the authors (n=1). In total, 13 articles were included, all published in English. A detailed diagram of the review process is presented in Figure 1.

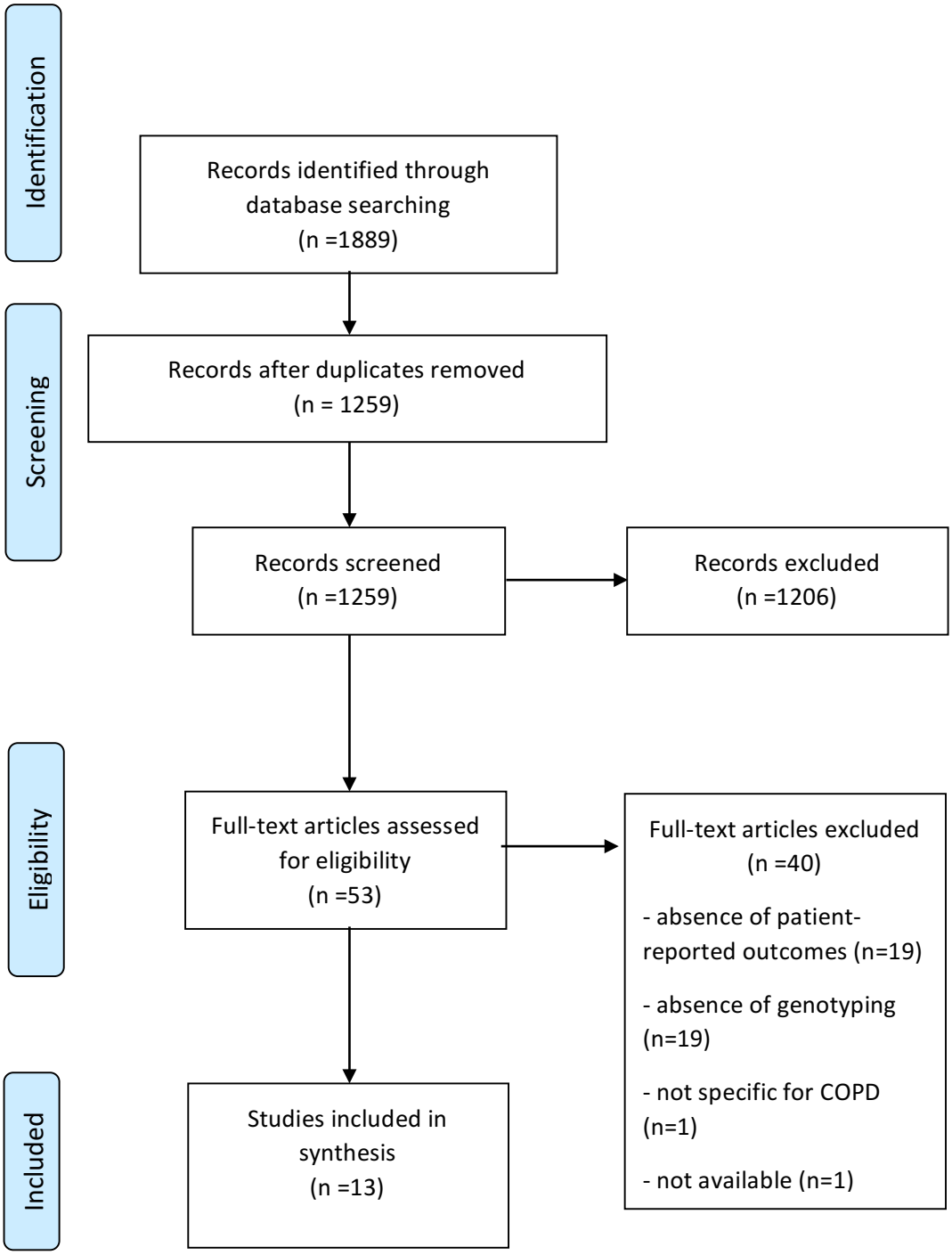

Figure 1-Flow diagram for study selection according to the preferred reporting items for systematic review and meta-analysis (PRISMA) guidelines.

Quality assessment

Articles scored between 39 and 55 on the Q-Genie checklist (12) (table 1). Six articles were classified as studies without control group (14-19), from which five presented of "good quality" and one presented "moderate quality". The remaining articles, were classified as "studies with control group", from which four had "moderate quality" and the remaining three had "good quality". Items with the lowest classification were the "selection and comparability of comparison groups" and "sample size and power". The agreement between the two independent reviewers was almost perfect (k=0.83; 95% CI 0.29-1; p=0.002).

Study characteristics

Study characteristics are presented in table 2. A total of 6520 patients with COPD with an age range of 63.2-71.8 years, mainly males (4638 males – 71,13%) participated in the 13 studies included. Studies designs were observational (n=9) (14, 15, 19-25) and pre and post intervention (n=4)(16-18, 26).

The most frequent genetic variants were the mannose-binding lectine (MBL2) gene variants (n=3) (14, 20, 22) and the ADRB2 polymorphisms (n=2) (16, 17). Other genetic variants observed were group component (GC) single nucleotide polymorphisms (SNPs) (n=1) (25), HHIP/CHRNA/FAM13A variants (n=1) (15), SERPINA 11478 G>A variant (n=1) (23), 25-hydroxyvitamin D receptor (VDR) polymorphisms (n=1) (24), CCL1 SNPs (n=1) (19), serotonin transporter gene variant (SLC6A4) (n=1) (21), HO-1 gene promotor polymorphism (n=1) (18) and EPHX1 polymorphisms (n=1) (26).

The PRO most assessed was the exacerbation frequency (n=7) (14, 15, 20, 22-25), followed by health-related quality of life (n=4) (16-18, 26), anxiety and depression (n=1) (21), exacerbation severity (19) and breathlessness, cough and sputum (n=1)(16). Exacerbation frequency was assessed using daily diaries (n=3)(23-25), phone calls (n=3)(14, 15, 20), questionnaires (n=1)(15)

and patient interviews (n=1)(22). Anxiety and depression were assessed using the Hospital Anxiety and Depression Scale (HADS)(n=1)(21), and health-related quality of life and respiratory health status using the St George Respiratory Questionnaire (SGRQ) (n=4) (16-18, 26). Exacerbation severity was also assessed with questionnaires (n=1)(19) and finally breathlessness, cough and sputum were assessed using the Breath Cough and Sputum scale (BCSS) (n=1)(16).

Table 1-Quality assessment score for selected studies based on the quality of genetic association studies (Q-Genie).

		Items											
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**Score**
Studies	**Bleecker et al,2012 (16)**	5	5	Na	5	4	5	6	5	6	5	5	51
	Ishii et al, 2014 (25)	5	4	3	6	3	3	3	5	4	4	5	45
	Ishii et al, 2011 (21)	6	4	5	4	2	5	2	3	4	4	5	44
	Lin et al, 2011 (22)	5	6	3	5	2	3	3	5	2	4	4	42
	Mandal et al, 2015 (14)	6	6	Na	3	2	6	1	4	3	5	6	42
	Pillai et al, 2010 (15)	4	6	Na	5	5	4	5	6	5	4	6	50
	Quint et al,2011 (23)	5	4	5	5	2	3	6	3	5	4	5	47
	Quint et al, 2012 (24)	4	5	3	4	5	4	2	6	3	4	4	44
	Takabatake et al, 2006 (19)	7	6	Na	6	5	5	3	6	6	5	6	55
	Umeda et al, 2008 (17)	5	4	Na	4	4	5	2	4	4	3	4	39
	Yang et al, 2003 (20)	6	6	4	5	5	5	3	5	5	5	6	55
	Zhang et al,2015(26)	5	5	5	4	4	5	4	6	4	4	4	50
	Zhang et al,2015 (18)	5	5	Na	4	4	4	5	6	4	4	5	46

Legend: 1- rationale for study; 2- selection and definition of outcome of interest; 3- selection and comparability of comparison group (if applicable); 4- technical classification of the exposure; 5- non-technical classification of the exposure; 6 other sources of bias; 7- sample size and power; 8- a priori planning of analysis; 9- statistical methods and control for confounding; 10- testing of assumptions and inferences for genetic analysis; 11- appropriateness of inferences drawn from results. All items have a maximum score of 7.
Na – not applicable

Synthesis of results

Genetic variants and exacerbation frequency

Two coding GC SNPs rs4588 and rs7041 (25), 5 SNP's and 7 haplotypes from MBL2 gene have been investigated for associations with exacerbation frequency (14, 20, 22). Only patients with C allele at the rs4588 polymorphism (C/C: 83 patients; A/C: 45 patients; A/A: 7 patients (p=0.0048)) (25), 3 MBL2 SNP's and 1 haplotype were found significantly more prevalent in frequent exacerbators (p<0.01) (14, 20, 22). HHIP, FAM13A and CHRNA3/5 SNPs were also assessed, however, only the rs13118928 SNP of the HHIP gene was found to be associated to previous and prospective exacerbations (Incidence Rate Ratio= 0.877; p=0.015 and IRR=0.906;p=0.024, respectively) (15). SERPINA1 11478 G>A variant (23) and 25-hydroxyvitamin D receptor (VDR) polymorphisms (24) were not associated with exacerbations frequency (α_1-antitrypsine: p=0.75; VDR polymorphisms: rs1544410: p=0.43; rs731236: p=0.64 rs2228570: p=0.87) (23, 24)

Genetic variants and exacerbation severity

The A allele from the rs2282691 SNP in CCL1 gene was found to be a risk allele for severity of exacerbation (OR 5.93; p=0.023) (19).

Genetic variants and depression

Only the rs3794808 SNP from the 5 SLC6A4 gene polymorphisms (rs3794808; rs140701; rs140700; rs2020939; rs2020936) was considered significantly associated with HADS depression score in patients with COPD (p=0.022)(21).

Genetic variants and health-related quality of life

The impact of HO-1 and EPHX1 polymorphisms on treatments with N-acetylcysteine (NAC) was assessed (18, 26). Better health-related quality of life, assessed with the SGRQ, was found in patients without the L allele (L-) of HO-1 gene, which is a $(Gt)_n$ polymorphism, than in those with

the L allele (L+) relative to the activity score of SGRQ (SGRQ activity score: Baseline: 46.2±14,5; 16 weeks: 46.3±11.0; 32 weeks: 46.7±12.2; 48 weeks: 47.4±15.5; p=0.02)(18). Additionally, patients having the slow activity group of the EPHX1 genotype (based on exon 3 polymorphism) also revealed better health-related quality of life than those having the fast activity group for the symptom score of SGRQ (Slow activity group: baseline: 40.9±12.3; after NAC: 37.8±13.1; Fast activity group: baseline: 41.7±13.5; after NAC 38.7±15.4; p<0.05) (26).

The impact of the Gly16Arg polymorphism of the ADRB2 gene on health-related quality of life HRQOL was also assessed. Significant differences were observed in all domains and total scores of SGRQ between both genetic groups (Arg/Arg and non- Arg/Arg), however only the impact and total scores were significantly different in patients with the Arg/Arg genotype (total score: -16.9 vs. -8.1, p=0.005; impact score: -19.8 vs. -2.2 p<0.001)(17). No significant associations were found when investigating impact of the ADRB2 polymorphism on treatment effect with budesonide/formoterol I(p=0.909) and II(p=0.648) on SGRQ(16).

Genetic variants and breathlessness, cough and sputum

No significant association was found between the Gly16Arg possible genotypes of ADRB2 and the scores for the BCSS scale (p>0.05)(16).

Table 2 -Patient reported outcomes, patient reported outcome measures and genetic associations assessed.

First author's name (Year)	Design	Population	Patient Reported Outcome (PRO)	Patient Reported Outcome Measure (PROM)	Genes of interest	Single Nucleotide Polymorphisms	Results
Bleecker et al, (2012) (16)	Intervention, pre/post study	Study I: n=1483 Gly/Gly: n=575 (370 Male, 64%, 63±9 yrs) Smoking status: Ex/current: 319/256 Pack-years: n.a. FEV_1 (%pred): 34.5±9.4 COPD grades (I/II/III/IV): n.a. Arg/Gly: n=685 (433 Male, 63%, 63±9 yrs) Smoking status: Ex/current: 396/289 Pack-years: n.a. FEV_1 (%pred): 34.4±9.2 COPD grades (I/II/III/IV): n.a. Arg/Arg: n=223 (139 Male, 62%, 63±10 yrs) Smoking status: Ex/current: 130/93 Pack-years: n.a. FEV_1 (%pred): 34.4±9.3 COPD grades (I/II/III/IV): n.a.	HRQOL; Symptoms	SGRQ BCSS	ADRB2	rs1042713	BCSS: Study I (p≥0.378); Study II (p≥0.133) SGRQ: Study I (p=0.909); Study II (p=0.648)

		Study II: n=1383 Gly/Gly: n=533 (373 Male, 70%, 64±9 yrs) Smoking status: Ex/current: 309/224 Pack-years: n.a. FEV_1 (%pred): 33.9±9.1 COPD grades (I/II/III/IV): n.a. Arg/Gly: n=635 (451 Male, 71%, 63±9 yrs) Smoking status: Ex/current: 373/262 Pack-years: n.a. FEV_1 (%pred): 34.6±9.7 COPD grades (I/II/III/IV): n.a. Arg/Arg: n=215 (133 Male, 62%, 63±9 yrs) Smoking status: Ex/current: 117/98 Pack-years: n.a. FEV_1 (%pred): 34.1±9.6 COPD grades (I/II/III/IV): n.a					
Ishii et al, (2014) (25)	Observational study	n=135 (127 Male, 94%, 69.3±7.9 yrs) Smoking status: Ex/current: 113/22 Pack-years: 74.5 ±47.6 FEV_1 (%pred): 57.8±20.3 COPD grades (I/II/III/IV): 22/61/41/11	Exacerbation frequency	Diary	GC	rs4588 rs7041	Exacerbation Frequency: **rs4588 SNP: p=0.0048;** rs7041 SNP: p=0.56.

Ishii et al, (2011) (21)	Observational study	n=247: COPD (228 Male, 92%, 69.7±8.1 yrs) Smoking status; Ex/Current: 207/40 Pack-years: 69.4±42.6 FEV_1 (%pred): 58.3±19.5 COPD grades(I/II/III/IV): n.a	Depressive symptoms	HADS	SLC6A4	rs3794808 rs140701 rs140700 rs2020939 rs2020936	**rs3794808, p(adjusted)/ Depression score HADS: Trend: 0.016; Genotype: 0.052** rs140701, p(adjusted)/ Depression score HADS: Trend: 0.093; Genotype: 0.246 rs140700, p(adjusted)/ Depression score HADS: Trend: 0.559; Genotype: 0.844 rs2020939, p(adjusted)/ Depression score HADS: Trend: 0.13; Genotype: 0.261 rs2020936, p(adjusted)/ Depression score HADS: Trend: 0.903; Genotype: 0.966
Lin et al, (2011) (22)	Observational study	n=84: Non-MBL -deficient genotypes (27 Male, 21%, 66.5±10.9 yrs) Smoking status: Ex/current: 60/24 Pack-years: 46 FEV_1 (%pred): 46 COPD grades(I/II/III/IV): 2/28/39/17 n=12: MBL-deficient genotypes (6 Male,50%,68.9±10.3 yrs) Smoking status: Ex/current: 10/2 Pack-years: 50 FEV_1 (%pred): 41 COPD grades(I/II/III/IV): 1/4/6/1	Exacerbation frequency	Patient interviews	MBL2	rs11003125 rs7096206 rs1800451 rs5030737 rs1800450	**Frequency of infective exacerbation (times) in:** **Non-MBL-Deficient genotypes (n=84): 3.52±0.78** **Total episodes: 296** **MBL-Deficient Genotypes (n=12): 4.75±1.22** **Total episodes: 57** **p<0.0001**
Mandal et al, (2015) (14)	Observational study	n=277 (190 Male, 84%, 67.8 ± 9.5 yrs) Smoking status: Ex/current: 203/74 Pack-years: Median 50 IQR (35-80) FEV_1 (%pred): 48.2±17.5	Exacerbation frequency	Contacting the patients	MBL2	HL (-550 G>C; rs11003125) YX (-221 G>C; rs7096206)	MBL2 haplotype: HYPA Infrequent exacerbation (n=87): Frequency: 0.282; Frequent exacerbation (n=85): Frequency: 0.346; p=0.13 MBL2 haplotype: LYQA

		COPD grades(I/II/III/IV): 0/129/104/40				PQ (+4 C>T; rs7095891) A/D (+223 C>T; rs5030737) A/B (+230 G>A; rs1800450) A/C (+239 G>A; rs1800451)	Infrequent exacerbation (n=75): Frequency: 0.243; Frequent exacerbation (n=53): Frequency: 0.215; p=0.49 MBL2 haplotype: LYPA Infrequent exacerbation (n=30): Frequency: 0.097; Frequent exacerbation (n=15): Frequency: 0.061; p=0.16 MBL2 haplotype: LXPA Infrequent exacerbation (n=71): Frequency: 0.230 Frequent exacerbation (n=50): Frequency: 0.303; p=0.50 MBL2 haplotype: LYPB Infrequent exacerbation (n=27): Frequency: 0.087; Frequent exacerbation (n=19): Frequency: 0.077; p=0.77 **MBL2 haplotype: HYPD** **Infrequent exacerbation (n=10): Frequency: 0.032** **Frequent exacerbation (n=21): Frequency: 0.085;** **p=0.01** MBL2 haplotype: LYQC Infrequent exacerbation (n=8): Frequency: 0.026; Frequent exacerbation (n=3): Frequency: 0.012; p=0.39

Pillai et al,
(2010) (15)
	Observational
	study
		n=1,609 (1086 Male, 67.5%, 63.8±
		7.1 yrs)
		Smoking Status: Ex/Current, %:
		64.5/35.5
		Pack-years: 50.9±28
			Exacerbation
			Frequency
				Retrospective
				exacerbations:
				questionnaires
					HHIP,
					FAM13A
					CHRNA3/5
						rs13118928
						rs8034191
						rs7671167
							Prior Exacerbations:
							rs13118928(HHIP): IRR: 0.877; 95% IC: 0.78-0.975;
							p=0.015
							rs8034191(CHRNA): IRR: 0.971; 95% IC: 0.869-1.084;
							p=0.598

		FEV_1: 48.1±15.6 COPD grades(I/II/III/IV): n. a		Prospective exacerbations: telephone calls.			rs7671167(FAM13A): IRR: 1.081; 95% IC: 0.978-1.195; p= 0.129 **Prospective exacerbations** **rs13118928(HHIP): IRR: 0.906; 95 IC: 0.832-0.987; p=0.024** rs8034191(CHRNA): IRR: 1.017; 95% IC: 0.930-1.113; p=0.709 rs7671167(FAM13A): IRR: 1.028; 95% IC: 0.943-1.22; p=0.528.
Quint et al, (2011) (23)	Observational study	n=204 (119 Male, 58%, 70.7 ±11.1 yrs) Smoking status: Ex/current: 152/52 Pack-years: 51.6±38.7 FEV_1 (l): 48.2±19.9 COPD grades(I/II/III/IV): 14/83/73/34	Exacerbation frequency	Diary	SERPINA1	11478G>A polymorphism	Exacerbation α_1-antitrypsin: (GG): Median=2.01 IQR (1.54-2.99); (GA/AA): Median=1.98 IQR (1.67-2.12); GG vs. GA/AA: p=0.75 baseline vs. exacerbation: (GG): p=0.87; (GA/AA): p=0.92
Quint et al, (2012) (24)	Observational study	n=97 (61 Male, 62.9%, 71.8±8.8 yrs) Smoking status: Ex/current: 72/25 Pack-years: 50.7±34.2 FEV_1 :(%pred): 50.3±19.7 COPD grades(I/II/III/IV): n.a	Exacerbation frequency	Diary	VDR	rs1544410 rs731236 rs2228570	rs1544410(Bsml): Frequent exacerbators (n=28): 3 (10.7%) – BB genotype; 12 (42.9%) – Bb genotype; 13 (46.4%) – Bb genotype. Infrequent exacerbators (n=68): 15 (22.1%) – BB genotype; 26 (38.2%) – Bb genotype; 27 (39.7%) – Bb genotype p=0.43 rs731236(TaqI): Frequent exacerbators (n=28):

							10 (38.5%) – TT genotype; 13 (50%) – Tt genotype; 3 (11.5%) – Tt genotype. Infrequent exacerbators (n=68): 24 (36.4%) – TT genotype; 29 (43.9%) – Tt genotype; 13 (19.7%) – Tt genotype p=0.64 rs2228570(FokI): Frequent exacerbators (n=28): 10 (35.7%) – FF genotype; 14 (50,0%) – Ff genotype; 4 (14.3%) – Ff genotype. Infrequent exacerbators (n=68): 21 (30.9%) – FF genotype; 38 (55.9%) – Ff genotype; 9 (13.2%) – Ff genotype p=0.87
Takabatake et al, (2006) (19)	Observational study	n=276 (276 Male, 100%, 72.9 ± 1.6 yrs) Smoking status: Ex/current: 276/0 Pack-years: n.a. FEV_1: (%pred) n.a. COPD grades(I+II/III+IV): n.a.	Exacerbation severity	Questionnaire	CCL1	rs2282691	Kaplan-Meier. Genotype: AA; No. of patients:60 No. of events: 9 Survival Time, mo (95% CI): 28 (27-30) Log-Rankk Statistic: AA vs AT: 1.51 (0.2189) **Genotype: AT; No. of patients: 132** **No. of events:12** **Survival Time, mo (95% CI): 29 (28-30)** **Log-Rankk Statistic: AA vs TT: 7.67 (0.0056)** Genotype: TT; No. of patients: 84

							No. of events:2 Survival Time, mo (95% CI): 30 (29-30) Log-Rankk Statistic: AT vs TT: 3.54 (0.0600) Cox Proportional Hazards Regression Model TT: n=84; p=n.a.; OR (95% CI) = 1 AT: n=132; p=0.066; OR (95% CI) = 4.09 (0.91-18.32) **AA: n=60; p=0.023; OR (95% CI) = 5.93 (1.28-27.48)**
Umeda et al, (2008) (17)	Intervention pre/post study	n= 44: n=22: Arg/Arg (21 Male, 95%, 73±8 yrs) Smoking status: Ex/current: 21/1 Pack-years: n.a FEV_1: (%pred) Median 50.7 Interquartile range (38.1-67.2) COPD grades(I/II/III/IV): n.a n=22: non-Arg/Arg (19 Male, 86%, 68±8 yrs) Smoking status: Ex/current: 21/1 Pack-years: n.a FEV_1 (%pred) Median 63.9 Interquartile range (42.3-89.0) COPD grades(I/II/III/IV): n.a	Quality of life	SGRQ	ADRB2	rs1042713	**Total Score:** **Arg/Arg: -16.9; non-Arg/Arg: -8.1** **P=0.05** **Impact score:** **Arg/Arg: -19.8; non- Arg/Arg: 22** **p<0.001**

Yang et al, (2003) (20)	Observational study	n=82 (52 Male,63 %,69±8 yrs) Smoking status: Ex/current: n.a/n. a Pack-years: Median 54 IQR (39-66) FEV_1:(%pred): 41±14 COPD grades(I/II/III/IV): n.a	Exacerbation frequency	Telephone call	MBL2	rs1800450	**OR 4.9; 95% IC: 1.7-14.4; p=0.0037,** **$p_{corrected}$= 0.011**
Zhang et al, (2015) (26)	Intervention pre/post study	n=219 (194 Male, 88.6%,70.2±7.2 yrs) Smoking status: n.a. Pack-years: 28.1±7.2 FEV_1:(%pred): 51.8±15.9 COPD grades(I/II/III/IV): n.a	Respiratory Health status	SGRQ	EPHX1	rs1051740 (slow allele) rs2234922 (fast allele)	Total score Slow activity group: Baseline: 36.9±12.1; After NAC: 35.1±12.7 p>0.05 Fast activity group: Baseline: 38.0±13.1; After NAC: 35.8±14.8 p>0.05 **Symptom score** **Slow activity group:** **Baseline: 40.9±12.3; After NAC: 37.8±13.1** **p=0.033** Fast activity group: Baseline: 41.7±13.5; After NAC: 38.7±15.4 P>0.05 Activity score Slow activity group: Baseline: 47.2±12.1; After NAC: 45.8±12.8 p>0.05 Fast activity group: Baseline: 48.7±13.3; After NAC: 49.1±14.0 p>0.05 Impact score Slow activity group:

							Baseline: 26.3±10.9; After NAC: 24.9±11.5 p>0.05 Fast activity group: Baseline: 28.1±11.7; After NAC: 26.5±12.1 p>0.05
Zhang et al, (2015) (18)	Intervention pre/post study	n=368 L+: n=154 (140 Male, 90.9%, 68.7±6.3 yrs) Smoking status: Ex/current: 123/31 Pack-years: 24.5±6.6 FEV_1: (%pred): 56.4±14.8 COPD grades(I/II/III/IV): n.a L-: n=214 (193 Male,90.2%,69.3±5 yrs) Smoking status: Ex/current: 170/44 Pack-years: 24.7±5.6 FEV_1: (%pred) 56.6±19.2 COPD grades(I/II/III/IV): n.a	Respiratory health status	SGRQ	HO-1	(GT)n polymorphism:	Total score Baseline – L+ group:35.0±11.6; L- group: 35.1±10.7 16 weeks – L+ group:35.7±9.6; L- group: 35.9±11.3 32 weeks – L+ group:36.2±11.2; L- group:36.4±12.4 48 weeks – L+ group:37.4±10.4; L- group: 37.8±9.0 p= 0.18 **Activity score** **Baseline – L+ group:46.7±13.9; L- group:46.2±14.5** **16 weeks – L+ group:46.5±14.1; L- group: 46.3±11.0** **32 weeks – L+ group:46.8±8.3; L- group: 46.7±12.2** **48 weeks – L+ group:46.3±11.5; L- group: 47.4±15.5** **p= 0.02** Symptom score Baseline – L+ group:40.5±10.8; L- group: 40.1±12.3 16 weeks – L+ group:40.8±8.4; L- group: 40.8±15.1 32 weeks – L+ group:41.1±6.9; L- group: 41.6±10.3 48 weeks – L+ group:41.7±13.7; L- group: 41.6±9.7 p= 0.86 Impact score Baseline – L+ group:25.6±10.1; L- group: 26.0±11.4

	16 weeks – L+ group:25.9±7.1; L- group: 26.5±8.6 32 weeks – L+ group:25.7±11.3; L- group: 26.9±10.5 48 weeks – L+ group:25.9±8.9; L- group: 26.7±13.4 p= 0.09

Legend: All values are presented as mean ± standard error, unless otherwise stated.

M- male; yrs- years; SNP- single nucleotide polymorphism; FEV_1- forced expiratory volume in 1 second; l- liters; %pred- % predicted; COPD- chronic obstructive pulmonary disease n.a- not applicable; SE- standard error; IRR- Incidence rate ratio; IC- Confidence interval; OR- Odds ratio; SGRQ- St. George Respiratory Questionnaire; BCSS- Breathlessness, cough and sputum scale; HADS - Hospital anxiety and depression scale. ´

Significant results presented in bold.

Discussion

This is the first systematic review to explore associations between genetics and PROs in patients with COPD. The 13 studies included reported on 12 genetic variations positively associated with 5 distinct PROs, i.e., exacerbation frequency and severity, depression, health related quality of life and symptoms (breathlessness, cough and sputum).

Most studies (n=7/13) assessed the association of specific genetic variants with exacerbation frequency. This is an important remark since the frequency of exacerbations is strongly associated with patients' functional and physiological deterioration (27), reduced health related quality of life (28) and substantial morbidity and mortality (29). MBL2 was the gene mostly associated with frequency of exacerbations (3/13) (14, 20, 22). Several polymorphisms of MBL2 gene play important roles in the innate immunity as it encodes for mannose-binding lectine. The mannose-binding lectine is a pattern-recognition receptor that binds to the sugar structure presented in various microorganisms(30). Specific polymorphisms of the MBL2 gene have been found responsible for causing a decreased production of MBL (MBL-deficient genotype), and this has been associated with an increased risk of exacerbations. In fact, high MBL levels presented in serum have been associated with increased survival in COPD (14). Thus, MBL2 polymorphisms seem to be promising biomarkers to detect those with more susceptibility to exacerbations and good candidates for assessment with PRO-based approaches.

Polymorphisms in the GC and VDR genes causing deficits of vitamin D (associated with several comorbidities, such as osteoporosis or skeletal muscle dysfunction, in patients with COPD(31)), have also been connected to the frequency of exacerbations. Nevertheless, a careful interpretation of the literature is needed since both significant and non-significant associations between GC polymorphisms rs4588 and rs7041 or VDR (Bsm, Taql, Fokl) polymorphisms with frequency of exacerbations and lack of vitamin D have been reported (25, 32, 33). Additionally,

many non-genetic factors may also lead to vitamin D deficiency, namely the absence of sun exposure, vitamin D retention on body fat or other social/cultural factors(31). Therefore, future studies are yet needed to enhance our understanding of the relationship between these polymorphisms, vitamin D deficiency and PRO in COPD.

Exacerbation severity has been significantly associated with a CCL1 allele for rs2282691 in one study (19). However, the authors' definition of exacerbation severity can be arguable, as they used death as endpoint. It is known that the severity of an exacerbation is not defined by mortality but rather by symptoms and number and length of hospitalizations in the most severe cases(34). Since the authors have recorded patients' main symptoms, it would be interesting to assess if the reported A allele for rs2282691 was also associated with those and if the T allele actually conferred protection to acute exacerbations of COPD, as suggested. Other option would be to explore the correlations between rs2282691 variant with specific instruments, such as the Exacerbations of Chronic Pulmonary Disease Tool (EXACT-PRO)(35) to assess the severity of exacerbations. This analysis would be essential to identify patients with higher predisposition for more severe exacerbations which would allow them to be targeted for more timely and directed monitoring and intervention.

Depression was found to be associated with the rs3794808 variant, affecting the SLC6A4 gene(21). It is known that depression presents a strong linkage to nicotine dependence(36) and SLC6A4 is strongly associated with the pathophysiology of tobacco use, namely at the level of serotonin reuptake. Therefore, it would be expected that specific genetic variants of this gene would play an important role on nicotine dependence and consequently depression in ex/current smokers(37). However, different SNPS of the SLC6A4 gene (38) and other genes such as THSD4, CHRNA, CYP2A6(3) have also been associated with depression in COPD. Thus, it would be valuable to confirm those associations populations patients with COPD with different characteristics, such as smokers and non-smokers, to decide if future therapies should take

these genes into consideration. Currently, the most effective therapy to combat anxiety and depression in patients with COPD is pulmonary rehabilitation (PR), which has been shown to be significantly effective by reducing the levels of depression and anxiety symptoms in patients with this disease (39). However, PR is an expensive therapy and access to it is highly limited (40). Therefore, genetics may be used to signal priority patients to PR, and thus optimize human and financial resources in managing COPD.

Four studies investigated ADRB2(16, 17), Ho-1(18) and EPHX1(26) association with health related quality of life. The Gly16Arg polymorphism of ADRB2 has been indicated as a risk factor for COPD(41). However, different results regarding its association with health-related quality of life emerged from our systematic review. Umeda et al., showed that patients with COPD and the Arg/Arg genotype presented better health related quality of life in treatments with tiotropium(17) whereas no significant associations was found in the study of Bleecker et al. for the same polymorphism and budesonide/formoterol treatment (16). The most obvious explanation is the substance used in the treatments, since other studies using other LABAs (long-acting b2-agonists) and LAMAs (long-acting muscarinic antagonists) have also presented no associations (42). As for Ho-1 and EPHX1 polymorphisms, both genes presented significant associations with SGRQ activity and symptoms sub-scores in patients with COPD that were treated with N-acetylcysteine (NAC)(18, 26). Pharmacogenetics studies are of significantly importance, since they investigate how genes affect a patient's response to drugs. This knowledge facilitates health-care by identifying patients that will respond differently to treatments. Future studies assessing health related quality of life may also include these genetic variants reported as being protective against COPD(43, 44), since they may play an important role on patients' quality of life.

PROs are increasingly being understood as excellent instruments to translate a range of outcomes that spirometry cannot express, such as symptoms and patients' perspective of

treatment(45). However, there is a massive number of PROMs (patient-reported outcomes measures) that were not found in this systematic review, and yet allow to assess other fundamental PROs such as mood, social and sexual life (6). Also, it was shown that genetics play a key role not only in the predisposition to the disease but also in common COPD-related comorbidities. Thus, further studies should be conducted to re-enforce the present knowledge and to assess the genetic influence on other dimensions of patients' lives.

Limitations

This review has some limitations that need to be acknowledged. Firstly, the definition of PROs (although published) has not been used as a primary outcome in some of the included studies. Thus, few studies gave emphasis to it, giving priority to clinical outcomes which may have led to significant loss of studies and information. However, to minimize this problem we performed a meticulous choice of keywords to diminish the number of missed studies. Secondly, the constant changes in the definition of exacerbation in the GOLD(1) may also result in loss of studies over the years. We overcome this drawback by enclosing studies which include participants that reported exacerbations independently of the definition used at that time. Thirdly, studies used different methodologies to assess similar or different PROs and consequently, prevented the realization of a meta-analysis.

Conclusion

This was the first systematic review to explore associations between genetics and PROs in COPD. Although a limited number of PROs have been successfully related to genetic variations, findings suggest that a significant association between specific genetic variants and the frequency and severity of exacerbations, health-related quality of life and depressive symptoms may exist.

Thus, further research is needed to confirm these results and to assess the possibility of association of other genetic variants with other PROs in patients with COPD, since this may enhance our understanding and management of this disease.

Conflicts of interest

The authors declared no conflicts of interest.

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Appendix II: Targeted gene sequencing panel

Table S 1- Targeted gene sequencing panel for microarray genotyping

Code	Gene	rs code	Chromosome	Location	Functional consequence	Allele
1	ABCC1	rs4148382	16	-	-	A/G
2		rs212093	16	16237704	-	A/G
3		rs35621	16	-	intron variant	C/T
4		rs1045642	16	-	synonymous codon	A/C/T
5	ABLIM3	rs3839234	5	148596694	intron variant	-/C
840	ACE	rs4646994	17	61565900	-	(289BPALU)/-
6	ADAM19	rs113897301	5	156929077	intron variant	-/T or AT/A
7		rs11134779	5	156936766	intron variant	C/T
8	ADAM33	rs612709	20	3652207	intron variant	A/G
9		rs3918396	20	3651765	Downstream variant 500B, missense, nc transcript variant	A/G
10		rs528557	20	3651742	Downstream variant 500B,nc transcript variant, synonymous codon	C/G
11		rs543749	20	3649679	intron variant	G/T
12		rs2787094	20	3649161	downstream variant 500B,nc transcript variant,utr variant 3 prime	C/G
13		rs2280090	20	3650205	Intron variant,missense,nc transcript variant	A/G

14		rs2280091	20	3650234	intron variant,missense,nc transcript variant	A/G
15	ADRB2	rs1042713	5	148206440	missense	A/G
16		rs1042714	5	148206473	missense	C/G/T
17		rs1042717	5	148206646	synonymous codon	A/C/G
18		rs1042718	5	148206917	synonymous codon	A/C
19	AGER	rs2070600	6	32151443	missense,nc transcript variant	A/G
20	ADGRG6	rs9399401	6	142668901	intron variant	C/T
21	AHNAK	rs2509961	11	62310909	intron variant	A/G/T
22	AQP5	rs3736309	12	50358054	downstream variant 500B, intron variant, upstream variant 2KB	A/G
23		rs2878771	12	50352393	intron variant,utr variant 3 prime	C/G
24		rs296763	12	50363014	intron variant,nc transcript variant	C/G
25	ARL15	rs2441026	5	53444498	Intron variant	C/T
26	ARMC2	rs2798641	6	109268050	intron variant,nc transcript variant	C/T
27		rs2806356	6	109266255	intron variant	C/G/T
28	BCL2	rs1564483	18	60794654	utr variant 3 prime	A/G
29		rs4987835	18	60806215	intron variant	A/G
30		rs1531697	18	60806606	intron variant	A/T

31		rs3943258	18	60813636	intron variant	A/G
32	BICD1	rs10844154	12	32380501	intron variant	A/C/T
33	C1GALT1	rs10246303	7	7286445	utr variant 3 prime	A/T
34	C5orf56	rs7713065	5	131788334	intron variant	A/C
35	C10orf11	rs11001819	10	78315224	intron variant	A/G
36	CACNA2D3	rs1458979	3	55150677	-	A/G
37	CASC20	rs6140050	20	6632901	-	A/C
38	CAT	rs1001179	11	34460231	upstream variant 2KB	A/G
39	CCDC38	rs1036429	12	96271428	intron variant	C/T
40		rs7957346	12	96260474	downstream variant 500B	A/C
41	CCDC101	rs17707300	16	28593347	intron variant	A/G
401	CCL1	rs2282691	17	32688309	intron variant	A/T
42	CCL5	rs2107538	17	34207780	intron variant, upstream variant 2KB	C/T
43		rs2280788	17	34207405	intron variant, upstream variant 2KB	C/G
44		rs2280789	17	34207003	intron variant, nc transcript variant	C/T
45	CDC123	rs7068966	10	12277992	intron variant	C/T
46	CD14	rs2569190	5	140012916	intron variant, utr variant 5 prime	A/G

47	CD40	rs1883832	20	44746982	nc transcript variant, utr variant 5 prime	C/T
48	CD86	rs1129055	3	121838319	missense	A/G
49	CDC6	rs2077464	17	38446564	intron variant	C/T
50		rs13706	17	38457151	missense	A/C/G
51		rs7217852	17	38470021	Intron variant	A/G
52		rs9904270	17	38479831	intron variant, upstream variant 2KB	C/T
53	CDC7	rs1192404	1	92068967	-	A/G
54	CDH13	rs4783244	16	82662268	intron variant	G/T
55		rs12922394	16	82672327	intron variant	C/T
56	CDKN1A	rs1801270	6	36651971	missense	A/C/T
57	CDON	rs567508	11	126008910	intron variant	A/C/T
58	CELSR1	rs9615358	22	46788781	intron variant	A/G/T
59	CFDP1	rs2865531	16	75390316	intron variant	A/T
60		rs7186831	16	75473155	-	A/G
61	CHRM3	rs6688537	1	239850588	intron variant	A/C
62	CHRNA3/5	rs1051730	15	78894339	nc transcript variant, synonymous codon	C/T
63		rs8034191	15	78806023	intron variant	C/T
64		rs578776	15	78888400	intron variant, utr variant 3 prime	C/T

65		rs16969968	15	78882925	intron variant, missense,nc transcript variant	A/G
66		rs6495309	15	78915245	downstream variant 500B, upstream variant 2KB	C/T
67		rs12910984	15	78819627	intron variant	A/G
68	CHRNB4	rs56218866	15	78922229	intron variant,missense, utr variant 5 prime	A/G
838	CLCA1	rs2145412	1	86939130	missense	A/C/T
839		rs1321694	1	86947975	synonymous codon	A/T
69	CISD3	rs11658500	17	36886828	intron variant	A/G
70	CNTN4	rs41526344	3	2985142	intron variant	C/T
71	COL4A3	rs11677877	2	228131169	intron variant, missense,nc transcript variant	A/G
72	CRP	rs1205	1	159682233	utr variant 3 prime	C/T
73	CSF2	rs25882	5	131411460	missense	C/T
74	CSF3	rs1042658	17	38173902	nc transcript variant, utr variant 3 prime	C/T
75		rs2227319	17	38170845	upstream variant 2KB	A/G
76		rs2227316	17	38170007	upstream variant 2KB	C/T
77	CTLA4	rs231775	2	204732714	missense	A/G/T
78		rs926169	2	204722752	-	C/G/T
79		rs11571316	2	204731089	upstream variant 2KB	C/T
80		rs3087243	2	204738919	downstream variant 500B	A/G

81		rs231725	2	204740675	-	A/G
82	CTSS	rs7534124	1	150739073	upstream variant 2KB	C/T
83		rs35989725	1	150738967:150738968	-	(CA)11/12
84		rs16827671	1	150738759	upstream variant 2KB	C/T
85		rs34495036	1	150738484:150738487	upstream variant 2KB	-/TCCC
86		rs3754212	1	150738200	utr variant 5 prime	C/T
87		rs1136774	1	150738197	utr variant 5 prime	A/G
88	CYBA	rs9932581	16	88718353	upstream variant 2KB, utr variant 3 prime	C/T
89		rs4673	16	88713236	missense	C/T
90	CYFIP2	rs10515750	5	156810072	intron variant	C/T
91	CYP1A1	rs1048943	15	75012985	missense	A/C/G/T
92		rs464624	5	102103919	intron variant	C/T
93	CYP1A2	rs762551	15	75041917	intron variant	A/C
94		rs35694136	15	75039613	upstream variant 2KB	-/T
95	CYP2A6	rs12459249	19	41339896	-	C/T
96	CYP2E1	rs2031920	10	135339845	nc transcript variant, upstream variant 2KB	C/T
97	CYP2R1	rs11819875	11	14917297	utr variant 5 prime	G/T
98	DNAH5	rs114929486	5	13887980	intron variant	A/G

99	DNLZ	rs10870202	9	139257411	intron variant	C/T
100	DSP	rs2076295	6	7563232	intron variant	G/T
101	EDN1	rs5370	6	12296255	missense	G/T
402	EDNRB	rs5351	13	78475313	intron variant, synonymous codon	A/C/G
102	EEFSEC	rs2955083	3	127961178	Intron variant	A/T
103	EFCAB5	rs62070270	17	28263980	Intron variant	A/G
104	ELN	rs41511151	7	73482987	missense	A/G
105	EPHX1	rs2234922	1	226026406	missense	A/G/T
106		rs1051740	1	226019633	missense	C/T
107	F2R	rs2227744	5	76010349	upstream variant 2KB	A/G
108	FAM13A	rs7671167	4	89883979	Intron variant	C/T
109		rs2045517	4	89870964	intron variant, upstream variant 2KB	C/T
110		rs2609264	4	89828080	Intron variant	A/G
111		rs2609261	4	89835485	Intron variant	C/T
112		rs2609260	4	89836819	Intron variant	A/G
113		rs6830970	4	89777081	Intron variant	A/G
114		rs2869966	4	89869078	intron variant	C/T
115		rs2869967	4	89869332	Intron variant	C/T

116	FAM19A2	rs348644	12	62463703	Intron variant	G/T
117	FGD6	rs113745635	12	95554771	Intron variant	C/T
118	FGF7	rs10519225	15	49720778	Intron variant	A/G
119	FGF10	rs2973644	5	44384183	Intron variant	A/C/T
120		rs10473352	5	44308252	Intron variant	A/G
121	FZD8	rs663700	10	35916372	-	A/G
122	GALNT13	rs6751439	2	154731287	Intron variant	A/G
123	GC	rs7041	4	72618334	intron variant, missense	G/T
124		rs4588	4	72618323	intron variant, missense	A/C/T
125		rs17467825	4	72605517	intron variant	A/G
126		rs1155563	4	72643488	Intron variant	C/T
127	GCLC	rs17883901	6	53410037	upstream variant 2KB	A/C/T
128	GCLM (DNTTIP2)	rs2235970	1	94345137	upstream variant 2KB	C/T
129	GDNF-AS1	rs10461985	5	37874409	intron variant	A/G
130	GLIS3	rs7872188	9	4124377	intron variant	C/T
131	GSTCD	rs10516526	4	106688904	intron variant	A/G
132		rs11727735	4	106631870	intron variant, upstream variant 2KB	A/G
421	GSTM1	Null genotype				

133	GSTO1	rs4925	10	106022789	intron variant, missense	A/C
134	GSTO2	rs156697	10	106039185	intron variant, missense	C/T
135		rs156699	10	106053641	intron variant	C/T
136	GSTP1	rs1695	11	67352689	missense	A/G
137		rs1138272	11	67353579	missense	C/T
420	GSTT1	GSTT1 null genotype				
138	HCRTR1	rs1056526	1	32084904	synonymous codon	C/T
139	HDAC4	rs12477314	2	239877148	-	C/T
140	HHIP	rs13118928	4	145486389	-	A/G
141		rs12504628	4	145436324	intron variant	C/T
142		rs1828591	4	145480780	intron variant	A/G
143		rs1980057	4	145485738	-	C/T
144		rs10519717	4	145480340	intron variant	C/T
145		rs11938704	4	145443370	intron variant	A/C
146		rs10013495	4	145505638	-	C/T
147	HLA-DQB1	rs114229351	6	32648418	-	C/T
403	HMOXI	rs3074372	22	35776887:35776888	upstream variant 2KB	-/GT
148	HTR4	rs3995090	5	147845815	Intron variant	A/C

149		rs11168040	5	139711031	-	A/G
150		rs6889822	5	147846707	intron variant	A/G
151	IDH3B	rs6107100	20	2644685	intron variant	A/C/T
152	IFIT3	rs140549288	10	91099466	intron variant, missense	C/G
153	IFNG	rs2069732	12	68554461	upstream variant 2KB	C/T
154		rs2069707	12	68554288	upstream variant 2KB	C/G
155		rs2430561	12	68552522	intron variant	A/T
156		rs1861493	12	68551196	intron variant	A/G
157		rs2069718	12	68550162	intron variant	A/C/T
158		rs2069727	12	68548223	downstream variant 500B	A/G
159	IL1A	rs1800587	2	113542960	utr variant 5 prime	C/G/T
160	IL1B	rs1143634	2	113590390	synonymous codon	C/T
161		rs16944	2	113594867	upstream variant 2KB	A/G
807	IL1RN	rs2234663	2	113888106:113888191	-	(ATCCTGGGGAAAGTGAGGGAAATATGGACATCACATGGAACAACATCCAGGAGACTCAGGCCTCTAGGAGTAACTGGGTAGTGTGC)2/3/4/5/6
162	IL2	rs2069762	4	123377980	upstream variant 2KB	G/T
163	IL27	rs153109	16	28519096	intron variant,upstream variant 2KB	C/T
164		rs17855750	16	28515228	missense	G/T

165	IL4	rs2070874	5	132009710	utr variant 5 prime	C/T
404		rs2243248	5	132008644	upstream variant 2KB	G/T
405	IL4R	rs1801275	16	27374400	downstream variant 500B, missense	A/G
166	IL6	rs1800795	7	22766645	intron variant, upstream variant 2KB	C/G
167		rs1800797	7	22766221	intron variant, nc transcript variant, upstream variant 2KB	A/G
168		rs1800796	7	22766246	intron variant, nc transcript variant, upstream variant 2KB	C/G
169		rs2069825	7	22765337:22765338	nc transcript variant,upstream variant 2KB	-/CT/TC
170	IL6R	rs4129267	1	154426264	intron variant	C/T
171	IL8	rs4073	4	74606024	upstream variant 2KB	A/T
172	IL8RB	rs2230054	2	219000310	synonymous codon	C/T
173	IL10	rs1800871	1	206946634	upstream variant 2KB	C/T
174		rs1518110	1	206944861	intron variant	G/T
175		rs1800896	1	206946897	upstream variant 2KB	A/G
176		rs1878672	1	206943713	intron variant	C/G/T
177		rs3024496	1	206941864	utr variant 3 prime	C/T
178		rs3024498	1	206941529	utr variant 3 prime	A/G
179	IL12A	rs2243115	3	159706280	intron variant, upstream variant 2KB	G/T

180	IL13	rs2066960	5	131994435	intron variant	A/C
182		rs20541	5	131995964	missense	C/T
182		rs1295685	5	131996445	utr variant 3 prime	C/T
183		rs1800925	5	131992809	upstream variant 2KB	C/T
184	IL13RA1	rs2250747	X	117863748	intron variant	A/G
185	IL16	rs11556218	15	81598269	missense, nc transcript variant	G/T
186	IL17F	rs763780	6	52101739	missense	C/T
187	IREB2	rs2568494	15	78740964	intron variant	A/G
188		rs2656069	15	78745707	intron variant	A/G
189		rs12593229	15	78765290	intron variant	G/T
190		rs10851906	15	78774676	intron variant	A/G
191		rs13180	15	78789488	synonymous codon	C/T
192		rs1964678	15	78754000	intron variant	C/T
193		rs965604	15	78789223	intron variant	C/T
194	ITGA1	rs1551943	5	52195033	intron variant	A/G
195	KCNE2	rs9978142	21	35652239	-	A/G/T
196	KCNMB1	rs11739136	5	169810796	intron variant, missense	C/T
197		rs2301149	5	169805956	intron variant, missense	A/C/G

198		rs2656842	5	169805630	intron variant, utr variant 3 prime	G/T
199		rs314156	5	169805411	intron variant, utr variant 3 prime	C/T
200	KCNQ5	rs141651520	6	73670096:73670101	intron variant	-/TTCTAT
201	KEAP1	rs11085735	19	10602180	intron variant	A/C
202	LEP	rs7799039	7	127878783	upstream variant 2KB	A/C/G
817	LEPR	rs1137100	1	66036391	missense	A/G
818		rs1805096	1	66102257	synonymous codon	C/T
819		rs7531867	1	66107546	-	A/G
820		rs1892535	1	66097181	intron variant	C/T
821		rs6588153	1	66092017	downstream variant 500B,intron variant	A/T
822		rs1938484	1	66081282	intron variant	A/C
823		rs12564626	1	66056542	intron variant	A/G
824		rs10443259	1	66051350	intron variant	A/G
825		rs6691346	1	66046397	intron variant	A/G
826		rs4655680	1	66041469	intron variant	G/T
827		rs6702028	1	66019891	intron variant	C/T
828		rs1782763	1	66007900	intron variant	C/T
829		rs1171265	1	66003252	intron variant	A/G

830		rs1171271	1	65998790	intron variant	C/T
831		rs1782754	1	65993348	intron variant	A/G
832		rs1171274	1	65980838	intron variant	C/T
833		rs10889558	1	65976966	intron variant	A/G
834		rs1327121	1	65957337	intron variant	C/T
835		rs17412682	1	65952293	intron variant	C/T
836		rs2025804	1	65946121	intron variant	C/T
837		rs9436740	1	65891901	intron variant	A/T
203	LRP1	rs11172113	12	57527283	intron variant	C/T
204	LTA	rs909253	6	31540313	intron variant	C/T
205	LTBP4	rs2077407	19	41119829	synonymous codon	A/C/T
206		rs2303729	19	41111069	missense,utr variant 5 prime	C/T
207		rs1131620	19	41117869	missense	A/G
208		rs1051303	19	41118056	missense	A/G
209	LOC105377462	rs11100860	4	145479139	intron variant	A/G
210	LOC153910	rs262129	6	142853144	intron variant	A/G
211	LOC101928765	rs16825116	2	229406685	intron variant	A/G/T
212	LST1	rs28986170	6	31556156:31556157	intron variant	-/AA

213	MAGI2	rs10251504	7	77689888	intron variant	A/G
406	MBL2	rs11003125	10	54532014	intron variant,upstream variant 2KB	C/G
407		rs7096206	10	54531685	intron variant,upstream variant 2KB	C/G
408		rs1800450	10	54531235	missense	A/G
409		rs5030737	10	54531242	missense	C/T
410		rs7095891	10	54531461	intron variant,upstream variant 2KB	A/G
214	ME3	rs145729347	11	86442733	intron variant	C/G
215	MECOM	rs1344555	3	169300219	intron variant	A/G
216	MFAP2	rs2284746	1	17306675	intron variant	C/G
217	MICAL3	rs11704827	22	18450287	intron variant	A/T
218	MGA	rs72724130	15	41977690	intron variant	A/T
219	MIR146A	rs2910164	5	159912418	nc transcript variant	C/G
220	MIR169A2	rs11614913	12	54385599	nc transcript variant	C/T
221	MIR499A	rs3746444	20	33578251	intron variant,nc transcript variant	C/T
222	MMP1	rs1799750	11	102670496	intron variant,upstream variant 2KB	-/G
223	MMP2	rs243865	16	55511806	upstream variant 2KB	C/T
224	MMP9	rs3918242	20	44635976	upstream variant 2KB	C/T
225	MMP12	rs2276109	11	102745791	upstream variant 2KB	A/G

226		rs652438	11	102736642	missense	A/C/G
227	MMP14	rs1003349	14	23305663	upstream variant 2KB	G/T
228		rs1042703	14	23306048	missense	C/T
229		rs2236302	14	23312554	synonymous codon	C/G
230		rs2236307	14	23312923	synonymous codon	A/C/G/T
231	MMP15	rs12447804	16	58075282	intron variant	C/T
232	MN1	rs2283847	22	28181399	intron variant	A/C/G/T
233	MOCS3	rs7269297	20	49576664	missense,upstream variant 2KB	G/T
234	MSR1	rs3747531	8	16012648	missense	C/G/T
235	MSRB3	rs1494502	12	65824670	intron variant	A/G
236	MTCL1	rs647097	18	8808464	intron variant	A/G
237	MYPN	rs7095607	10	69957350	intron variant	A/G
238	NAT2	rs1799929	8	18257994	synonymous codon	C/T
239		rs1799930	8	18258103	missense	A/G
240		rs1208	8	18258316	missense	A/G
241		rs1799931	8	18258370	missense	A/G
242	NCR3	rs2857595	6	31568469	-	C/T
243	NFE2L2	rs2364723	2	178126546	intron variant	C/G/T

244	NFKB1B	rs2241704	19	39396335	intron variant	A/T
245	NOS3	rs1799983	7	150696111	missense	G/T
246		rs3918161	7	150690009	intron variant, upstream variant 2KB	C/T
247		rs1800779	7	150689943	intron variant, upstream variant 2KB	A/G
411		4B/4A	7	150694144:150694644	-	-
248		rs1549758	7	150695726	synonymous codon	C/T
249	NQO1	rs1800566	16	69745145	missense	C/T
250	NR3C1	rs6195	5	142779317	missense	A/G/T
251	NT5C3B	rs4796712	17	39987130	nc transcript variant,synonymous codon	A/C/T
252	NTSDC1	rs1052443	6	116568773	-	A/C
253	ODZ2	rs516732	5	165107100	-	C/T
254	OGG1	rs1052133	3	9798773	downstream variant 500B, intron variant,missense, utr variant 3 prime	C/G
255	OLIG3	rs473892	6	137951047		A/G
256	OR4X1	rs10838851	11	48286231	stop gained, synonymous codon	A/C/T
257	PARK2	rs577876	6	161923155	intron variant	A/G
258		rs6455728	6	161849789	intron variant	A/G/T
259		rs9346917	6	162914986	intron variant	C/T

260	PCDH9	rs17490056	13	66726903	-	C/T
261	PDE4D	rs829259	5	58267976	utr variant 3 prime	A/T
262		rs159497	5	58208218	-	A/G
263		rs16878037	5	59709752	intron variant	C/T
264	PLAUR	rs2302524	19	44156472	intron variant,missense	C/T
265		rs2283628	19	44163061	intron variant	C/T
266		rs740587	19	44196668	intron variant,upstream variant 2KB	C/T
267		rs11668247	19	44194362	intron variant	C/T
268		rs344779	19	44194362	-	G/T
269	PPARG	rs3856806	3	12475557	intron variant, synonymous codon, utr variant 3 prime	C/T
270	PPT2	rs10947233	6	32124424	-	C/G/T
271	PRDX5	rs9787810	11	64085298	upstream variant 2KB, utr variant 5 prime	C/T
272	PTCH1	rs16909859	9	98204792	downstream variant 500B	A/G
273	PTEN	rs701848	10	89726745	utr variant 3 prime	C/T
841		rs1903858	10	89653686	intron variant	C/T
274	PTGS2	rs20417	1	186650321	nc transcript variant, upstream variant 2KB	C/G
275	RAB4B	rs7937	19	41302706	intron variant,nc transcript variant,utr variant 3 prime	A/C/T
276		rs2604894	19	41292404	intron variant	C/T

277	RIN3	rs754388	14	93115410	intron variant	C/G
278	RARB	rs1529672	3	25520582	intron variant	A/C
279	SATB1	rs6577641	3	18397849	intron variant	C/T
800	SERPINA1	rs8004738	14	94856914	upstream variant 2KB,utr variant 5 prime	A/G
801		rs17751769	14	94856657	intron variant, upstream variant 2KB	A/G
802		rs709932	14	94849201	missense	A/G
803		rs11832	14	94843565	utr variant 3 prime	A/G
804		rs1303	14	94844843	missense	G/T
805		rs28929474	14	94844947	missense	A/G
806		rs17580	14	94847262	missense	A/T
280	SERPINA3	rs1800463	14	95081011	missense	A/C/T
281		rs17473	14	95085642	missense	C/G/T
282	SERPINE2	rs6754561	2	224839696	downstream variant 500B	C/T
283		rs6734100	2	224841995	intron variant	C/G
284		rs729631	2	224844919	intron variant	C/G/T
285		rs975278	2	224847707	intron variant	A/G/T
286		rs6436449	2	224848333	intron variant	C/T
287		rs7608941	2	224854068	intron variant	A/C

288		rs1371028	2	224879450	intron variant	C/T
289	SERPINA12	rs140198372	14	94953832	intron variant,splice acceptor variant	A/C
290	SETD7	rs17050782	4	140423134	intron variant	A/G
291	SFTPA1	rs1136450	10	81371729	intron variant,missense	C/G
292	STFTPA2	rs17886395	10	81318663	missense	C/G
293	SFTPB	rs3024791	2	85895704	intron variant,upstream variant 2KB	A/G
294		rs2118177	2	85890293	intron variant	C/T
295		rs2304566	2	85890758	intron variant	A/G
296		rs1130866	2	85893741	missense	C/T
297	SFTPD	mrs721917	10	81706324	missense	C/T
298		rs10887199	10	81702834	intron variant	C/T
299		rs2245121	10	81699238	intron variant	A/G
300		rs911887	10	81701523	intron variant	A/G
301		rs6413520	10	81706281	synonymous codon	C/T
302		rs7078012	10	81705433	intron variant	C/T
303		rs3088308	10	81697868	missense	A/T
304	SH3GL3	rs66650179	15	84261690	intron variant	-/A
305	SIRT1	rs7895833	10	69623057	-	A/C/G

306		rs2273773	10	69666598	synonymous codon	C/T
307		rs7069102	10	69663120	intron variant	C/G
308	SLC6A4	rs2020936	17	28550814	intron variant	C/T
309		rs3794808	17	28531793	intron variant	A/G
310	SLC11A1	rs3731865	2	219250003	intron variant	A/C/G
311		rs17235409	2	219259732	missense,nc transcript variant	A/C/G
312		rs1059823	2	219259844	nc transcript variant,utr variant 3 prime	A/G
313	SMAD3	rs28683050	15	67378336	intron variant	C/T
314	SMOC2	rs1402	6	168943376	intron variant	G/T
315		rs747995	6	168930816	intron variant	A/G
316	SOD2	rs2842958	6	160108425	intron variant	A/G
317		rs4880	6	160113872	missense,utr variant 5 prime	C/T
318	SOD3	rs1799895	4	24801834	missense,nc transcript variant	C/G
319		rs8192288	4	24796678	upstream variant 2KB	A/G/T
320		rs8192287	4	24796568	upstream variant 2KB	G/T
321	SOX5	rs11046966	12	23677692	-	C/T
322	SPAG17	rs200154334	1	118862071:118862072	-	-/AT
323	SPATA9	rs153916	5	95036700	intron variant	A/G

324	STAT1	rs13010343	2	191843445	intron variant	A/G
325	SUCLG2	rs1490265	3	67452043	intron variant	A/C
326	SVIL	rs3847402	10	30267810	-	A/G
327	TET2	rs2047409	4	106137033	intron variant	C/T
328	TBX3	rs35506	12	115500691	intron variant	A/T
329	TERT	rs2736100	5	-	intron variant	G/T
330	TGFB	rs1800469	19	41860296	downstream variant 500B,upstream variant 2KB	C/T
331		rs1800470 or rs1982073	19	41858921	missense	C/G/T
332		rs1800471	19	41858876	missense	C/G
333		rs11083616	19	41865643	intron variant	A/G
334		rs11466321	19	41854916	intron variant	C/G/T
335		rs2241712	19	41869756	intron variant, upstream variant 2KB, utr variant 5 prime	A/G
336		rs2241718	19	41829606	utr variant 3 prime	C/T
337		rs6957	19	41830606	utr variant 3 prime	A/G
808	TGFBR3	rs2296621	1	92163786	intron variant	A/C
809		rs2291477	1	92178335	intron variant	C/G
810		rs284170	1	92214628	intron variant	C/G/T
811		rs10874996	1	92300084	intron variant	A/G

812		rs2129972	1	92305250	intron variant	A/G
813		rs2634028	1	92320853	intron variant	A/C
814		rs2046737	1	92326636	intron variant	A/G
815		rs12727153	1	92331518	intron variant	A/C
816		rs1805110	1	92327045	missense,nc transcript variant	C/T
338	THSD4	rs8033889	15	71680080	intron variant	G/T
339		rs1441358	15	71612514	intron variant	G/T
340		rs12591467	15	71788387	intron variant	C/T
341		rs12899618	15	71645120	intron variant	A/G
342	TIMP1	rs4898	X	47444985	intron variant, synonymous codon, upstream variant 2KB	C/T
343		rs11551797	X	47445940	downstream variant 500B, intron variant, synonymous codon, upstream variant 2KB	C/T
344	TIMP2	rs2277698	17	76867017	synonymous codon	A/G
345		rs8179090	17	76921889	upstream variant 2KB	C/G
346	TLR4	rs4986791	9	120475602	missense	C/T
347	TLR9	rs187084	3	52261031	upstream variant 2KB	C/T
348	TMEM176A	rs2888674	7	150510915	-	A/G/T
349	TNF	rs1800629	6	31543031	upstream variant 2KB	A/G

350		rs769178	6	31547514	-	A/C
351		rs3091257	6	31546850	-	C/T
352		rs361525	6	31543101	upstream variant 2KB	A/G
353		rs1800630	6	31542476	downstream variant 500B, upstream variant 2KB	A/C
354		rs56036015	6	31543064	upstream variant 2KB	A/G
355	TNS1	rs2571445	2	218683154	missense	C/T
356	TP53	rs1042522	17	7579472	missense, upstream variant 2KB	A/C/G
357	TRPV4	rs12578401	12	110233500	intron variant	C/T
358		rs12579553	12	110235632	intron variant	A/G
359		rs16940583	12	110238265	intron variant	A/G
360		rs3742030	12	110252547	missense	C/G/T
361	TSLP	rs3806933	5	110406742	nc transcript variant, upstream variant 2KB	A/C/T
362		rs2289276	5	110407507	intron variant, upstream variant 2KB, utr variant 5 prime	C/T
363	TSPYL4	rs3749893	6	116571695	utr variant 3 prime	A/G
364	VDR	rs10735810 or rs2228570	12	48272895	missense	A/C/G/T
365		rs1544410	12	48239835	intron variant	A/G
412		rs731236	12	48238757	synonymous codon	C/T
366	VEGFR1	rs7326277	13	28876214	utr variant 3 prime	C/T

367	XRCC1	rs25487	19	44055726	missense	A/G
368	XRCC5	rs828922	2	216956668	intron variant	C/T
369		rs3821104	2	217057846	intron variant	C/T
370		rs207936	2	217040033	intron variant	C/T
371	ZGPAT	rs72448466	20	62363641:62363642	intorn variant	-/GT
372	ZKSCAN1	rs72615157	7	99635967	intron variant, nc transcript variant, utr variant 3 prime	A/C/G
373	ZKSCAN3	rs6903823	6	28322296	intron variant, upstream variant 2KB, utr variant 5 prime	A/G
374	ZMAT	rs13278529	8	40333321	-	G/T
375	WNT16	rs2707469	7	120976886	intron variant	C/T
376	WWOX	rs383362	16	79245820	missense,nc transcript variant,utr variant 3 prime	G/T
377		rs2857595	-	-	-	-
378		rs2571445	-	-	-	-
379		rs9978142	-	-	-	-
380		rs993925	-	-	-	-
381		rs12477314	-	-	-	-
382		rs9296092	-	-	-	-
383		rs950063	-	-	-	-

384		rs734556	-	-	-	-
385		rs10795108	-	-	-	-
387		rs8102683	-	-	-	-
388		rs11878604	-	-	-	-

Appendix III: Characteristics of the total 60 DNA samples selected for genotyping

Table S 2-Characteristics (DNA concentration and ratios from Denovix and DNA concentration from Qubit) of the 60 samples selected for microarray genotyping

Patient Code	Denovix Mean DNA conc (ng/µl)	Mean ± SD 260/280	Mean ± SD 260/230	Qubit Mean DNA conc (ng/µl)
AO_E4	37,784	1,75	1,47	30,6
AO_S24	22,049	2	1,32	11,3
AO_S3	30,015	1,86	1,38	23,4
AO_E22	10,459	1,86	1,99	12,2
AO_E46	7,999	1,8	0,89	7,2
AO_147	28,671	2,01	0,73	24,4
500	25,766	1,96	1.04	16,1
503	29,798	1,88	1,24	52,6
508	24,637	1,93	1,03	11,1
516	14,903	1,75	0,93	9,2
520	50,832	1,93	1,18	23,4
523	40,138	1,81	1,86	27,4
526	21,359	1,86	1,44	8,12
529	14,217	1,86	2,18	9,42
535	8,657	1,77	2,09	6,98
536	21,245	1,82	1,84	16,9
543	34,487	1,86	1,92	24,6
546	29,659	1,81	1,98	17,2
554	16,401	1,88	0,96	8,44
556	192,784	1,82	1,8	153
558	59,103	1,79	1,82	64
563	91,474	1,83	1,71	82
572	12,645	1,91	2,4	9,24
579	55,533	1,88	2,14	26,6
583	19,637	2,01	2,11	18,1
607	93,105	1,92	1,62	65
609	39,219	1,84	1,63	39,4
616	19,121	2,23	1,51	9,26
623	31,302	1,95	1,72	35,8

644	49,901	1,84	1,51	46,4
646	30,955	1,9	1,6	18,1
647	18,896	1,92	1,37	8,16
661	16,183	1,98	1,82	14,1
671	28,148	1,94	2,09	18,7
672	24,838	1,98	1,72	15,2
674	12,69	1,97	1,03	5,54
678	18,386	1,99	1,79	13,9
686	22,228	2,01	1,4	16,7
696	35,341	1,84	1,27	34,4
698	47,488	1,87	1,57	31,4
713	35,945	1,98	1,14	24,6
716	41,274	1,79	1,08	49,2
736	20,686	1,96	1,19	21,6
744	35,203	1,94	1,91	29,6
746	45,765	1,84	1,09	48,8
758	14,488	2,13	1,61	14,9
765	83,485	1,78	1,66	106
766	15,653	1,92	1,29	13,6
768	30,024	2,02	1,76	24,4
774	71,259	1,83	1,47	11,6
783	23,394	1,85	1,33	12,8
784	13,028	1,82	1	9,6
791	38,938	1,93	1,35	46,4
795	15,611	1,86	1	13
796	17,244	2,18	0,8	9,98
542	10,728	1,78	1,71	8,18
687	17,417	1,78	1,41	13,3
777	16,393	1,73	0,69	13
787	11,839	1,7	0,87	12,4
666	128,526	1,9	1,89	114

Appendix IV: Poster discussion (PA4713) at 2017 European Respiratory Society (ERS)

Genetic profile and patient-reported outcomes (PROs) in COPD: a systematic review

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Background

- Chronic Obstructive Pulmonary Disease (COPD) may impact differently on patients with similar lung function impairment[1], suggesting that factors other than lung function may influence patients' experience of the disease.
- Recent studies have found associations between genetic variations and patient-reported outcomes (PROs).
- PROs are defined as a set of health outcomes directly reported by patients.
- Identifying these associations might be fundamental to predict the disease progression and develop tailored interventions.

Aim

- This systematic review aimed to identify the genetic variations associated with PROs in COPD.

Methods

- **Search Strategy:** Pubmed, Scopus and Web of Science were searched until May 2017 (PROSPERO Reg. CRD42016041639). Additional searches were conducted scanning the reference list of the articles.
- **Eligibility Criteria:** Studies were eligible if included patients diagnosed with COPD and associated a genetic variant to one or more PROs.
- **Quality assessment:** 2 independent reviewers assessed the quality of studies using the Q-Genie checklist[2]. This checklist is composed of 11 questions, each with 7 possible answers from "1-poor" to "7-excelent"

Results

- 13 reporting on associations between 5 PROs and 12 genetic variants studies were included (Fig. 1). Quality of studies were rated as "good" (n=8) and "moderate" (n=5).

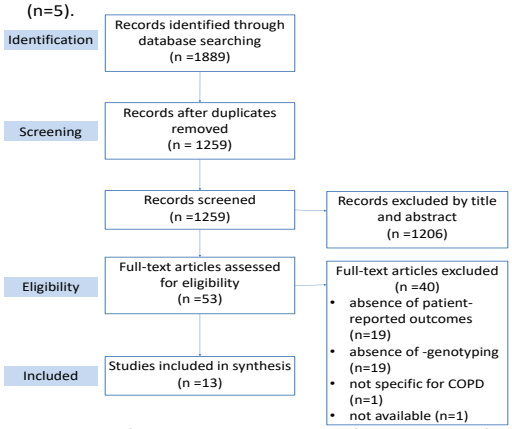

Fig. 1 –Flow diagram for study selection according to the preferred reporting items for systematic review and meta-analysis (PRISMA) guidelines.

Results

- The most reported PRO was frequency of exacerbations (n=7/13), which was often associated with MBL2 gene variants (n=3/7). Other PRO's reported were health-related quality of life (n=4/13), depressive symptoms (n=1/13), exacerbation severity (n=1/13) and breathlessness, cough and sputum (n=1/13), which were commonly associated with other gene variants (Fig. 2).

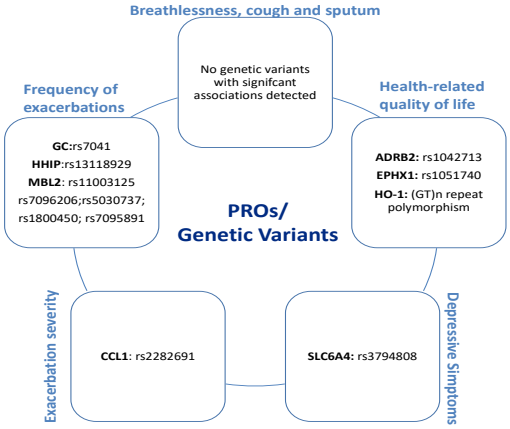

Fig. 2 – Significant correlations between PROs and specific genetic variants in COPD.

Conclusions

- A limited number of PRO's have been explored and related to genetic variations.
- Findings suggest significant associations between specific gene variants and the number/severity of exacerbations, depressive symptoms and health-related quality of life.
- Further research is needed to confirm these findings and assess the genetic influence on other dimensions of patients' lives, since it may enhance our understanding and management of COPD.

References

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Appendix V: Associations between SNPs and PROS/clinical outcomes (Results)

Table S 3-Associations between modified medical research council and genetic variants

SNP	Genotype	N	Median	IQR	Chi-Square	p-value*
rs10011792	CC	37	2.0000	1 to 3		
	CT	22	3.0000	1.75 to 3	1.342	.247
	TT	-	-	-		-
rs1042522	CC	5	2.000	1 to 3		
	CG	25	2.0000	1 to 3	.805	.669
	GG	30	2.5000	1.75 to 3		
rs1042713	CC	21	2.5000	2 to 3		
	TC	24	2.5000	1 to 3	1.260	.533
	TT	14	2.0000	1 to 3		
rs1042714	GG	30	2.0000	1 to 3		
	CG	24	3.0000	2 to 3	6.003	.050
	CC	5	2.0000	1.50 to 3		
rs1042717	CC	31	3.0000	1 to 3		
	CT	25	2.0000	1.25 to 3	.990	.610
	TT	4	1.5000	1 to 2.75		
rs10461985	GG	56	2.0000	1 to 3		
	GA	4	3.0000	.75 to 3	.127	.721
	AA	-	-	-		
rs1051303	CC	8	3.0000	1.25 to 3.75		
	CT	35	2.0000	1 to 3.0	1.962	.375
	TT	16	3.0000	1.25 to 3		
rs10516526	GG	50	3.0000	1 to 3		
	GA	10	2.0000	2 to 2.5	4.061	.044
	AA	-	-	-		
rs1051740	AA	41	3.0000	1.25 to 3		
	AG	16	2.0000	1 to 3	2.925	.232
	GG	2	1.5000	1 to -		
rs1059823	TT	14	2.5000	1 to 3		
	TC	26	3.0000	2 to 3	2.195	.334
	CC	20	2.0000	1 to 3		
rs10844154	TT	10	2.5000	1 to 3		
	TG	35	2.5000	1 to 3	.245	.885
	GG	15	2.0000	1 to 3		
rs11001819	GG	23	2.0000	1 to 3		
	GA	27	3.0000	2 to 3	2.254	.324
	AA	10	1.0000	1 to 3		
rs11046966	AA	30	3.0000	1 to 3		
	AG	23	2.0000	1.75 to 3	3.579	.167
	GG	7	1.0000	1 to 2		
rs11172113	AA	9	2.0000	1 to 3.5		
	AG	22	2.0000	1 to 3	3.832	.147
	GG	29	3.0000	2 to 3		
rs1129055	GG	37	2.0000	1.25 to 3		
	GA	19	3.0000	1 to 3	.323	.851
	AA	-	-	-		
rs1130866	TT	13	3.0000	2 to 3		
	TC	37	2.0000	1 to 3	.427	.808
	CC	10	2.5000	1 to 3		
rs1131620	CC	9	3.0000	1.50 to 3.50		
	CT	35	2.0000	1 to 3	1.660	.436
	TT	16	3.0000	1.25 to 3		
rs1138272	CC	53	2.0000	1 to 3		
	CT	5	3.0000	1.5 to 3.5	.789	.375
	TT	-	-	-		
rs1143634	GG†	36	2.0000	1 to 3		
	GA	19	3.0000	2 to 3	9.352	.009
	AA	5	3.0000	3 to 3.5		
rs1155563	CC	2	3.0000	-		
	CT	25	3.0000	1 to 3	2.357	.308
	TT	30	2.0000	1 to 3		
rs11556218	TT	46	2.0000	.2 to 3		
	TG	14	1.5000	1 to 3	1.549	.213
	GG	-	-	-		

rs11614913	CC CT TT	30 24 6	2.0000 2.5000 3.0000	1 to 3 1 to 3 2.50 to 3.25	2.895	.235
rs11677877	TT TG GG	53 6 -	2.0000 2.5000 -	1 to 3 1.75 to 3 -	.062	.803
rs11739136	GG GA AA	47 13 -	2.0000 2.0000 -	1 to 3 1.25 to 3 -	.007	.933
rs12477314	GG GA AA	39 19 2	3.0000 3.0000 2.0000	1 to 3 1 to 3 1 to -	.309	.857
rs12504628	TT TC CC	31 25 4	2.0000 3.0000 2.5000	1 to 3 2 to 3 1.25 to 3.75	4.124	.127
rs12899618	CC CT TT	46 14 -	2.0000 2.5000 -	1 to 3 2 to 3 -	.634	.426
rs12922394	GG GA AA	56 4 -	2.0000 1.5000 -	1 to 3 1 to 2.75 -	.956	.328
rs1303	TT TC CC	28 27 5	2.0000 3.0000 2.0000	1 to 3 1 to 3 1 to 2.5	1.252	.535
rs1800450	CC CT TT	44 15 -	2.0000 2.0000 -	1 to 3 1 to 3.25 -	.680	.712
rs5030737	GG GA AA	52 8 -	2.0000 2.5000 -	1 to 3 1 to 3 -	.005	.946
rs7041	GG GT TT	22 29 9	2.0000 3.0000 3.0000	2 to 3 1 to 3 2.50 to 2.50	6.549	.038

*Calculated using Kruskal-Wallis test

† Significant different from GA

SNP- Single Nucleotide Polymorphism

IQR- Interquartile range

Table S 4-Associations between frequency of exacerbations and genetic variants

SNP	Genotype	N	Median	IQR	Chi-Square	p-value*
rs10011792	CC	37	1.0000	0 to 3		
	CT	22	2.0000	0 to 3	.403	.526
	TT	-	-	-		
rs1042522	CC	5	2.0000	1 to 4		
	CG	25	1.0000	0 to 3	.701	.704
	GG	30	1.0000	0 to 2.25		
rs1042713	CC	21	2.5000	1 to 4.75		
	TC	24	1.0000	0 to 2	4.350	.114
	TT	14	1.5000	0 to 5.25		
rs1042714	GG	30	1.0000	0 to 3		
	CG	24	2.0000	1 to 3	2.233	.327
	CC	5	1.0000	0 to 2.5		
rs1042717	CC	31	1.0000	0 to 3		
	CT	25	2.5000	1 to 4.50	4.323	.115
	TT	4	1.0000	0.25 to 1		
rs10461985	GG	56	1.0000	0 to 3		
	GA	4	1.5000	0 to 5.25	.002	.964
	AA	-	-	-		
rs1051303	CC	8	0.5000	0 to 4.50		
	CT	35	1.0000	0 to 3	2.308	.315
	TT	16	2.5000	1 to 4.50		
rs10516526	GG	50	1.0000	0 to 3		
	GA	10	.0000	0 to 2	2.959	.085
	AA	-	-	-		
rs1051740	AA	41	1.0000	0 to 3		
	AG	16	1.5000	0 to 3	.010	.995
	GG	2	2.5000	0 to -		
rs1059823	TT	14	1.0000	0 to 5		
	TC	26	2.0000	1 to 4.50	4.506	.105
	CC	20	1.0000	0 to 2		
rs10844154	TT	10	2.5000	0.75 to 3		
	TG	35	1.0000	0 to 3	1.057	.589
	GG	15	1.0000	1 to 3		
rs11001819	GG	23	1.0000	0 to 3		
	GA	27	1.0000	0 to 3	.029	.985
	AA	10	1.5000	0 to 4.50		
rs11046966	AA	30	2.0000	1 to 3		
	AG	23	1.0000	0 to 5	4.022	.134
	GG	7	.0000	0 to 1		
rs11172113	AA	9	3.0000	1 to 5		
	AG	22	1.0000	0 to 2	4.471	.107
	GG	29	1.0000	0 to 3.75		
rs1129055	GG	37	1.0000	0 to 3		
	GA	19	1.0000	0 to 2	4.610	.100
	AA	-	-	-		
rs1130866	TT	13	1.0000	.5 to 2		
	TC	37	1.0000	0 to 3	.857	.652
	CC	10	2.0000	0 to 5.25		
rs1131620	CC	9	0.	0 to 4		
	CT	35	1.0000	0 to 3	2.861	.239
	TT	16	2.5000	1 to 4.50		
rs1138272	CC	53	1.0000	0 to 3		
	CT	5	3.0000	2 to 3	2.359	.125
	TT	-	-	-		
rs1143634	GG	36	1.0000	0 to 3		
	GA	19	2.5000	1 to 3.25	1.821	.402
	AA	5	3.0000	0 to 7.50		
rs1155563	CC	2	3.0000	1 to -		
	CT	25	1.0000	0 to 4	.844	.656
	TT	30	1.0000	0 to 3		
rs11556218	TT	46	1.0000	0 to 3		
	TG	14	1.5000	0 to 5.25	.063	.0.802
	GG	-	-	-		

rs11614913	CC	30	1.0000	0 to 2.50		
	CT	24	1.5000	1 to 3	4.335	.114
	TT	6	3.0000	0.75 to 7		
rs11677877	TT	53	1.000	0 to 3		
	TG	6	3.5000	0.75 to 5.25	1.964	.161
	GG	-	-	-		
rs11739136	GG	47	1.0000	0 to 3		
	GA	13	1.0000	0.25 to 2.75	.037	.847
	AA	-	-	-		
rs12477314	GG	39	1.0000	0 to 3.50		
	GA	19	1.0000	0 to 3	1.754	.416
	AA	2	1.0000	0 to 3		
rs12504628	TT	31	1	0 to 3		
	TC	25	2.0000	0 to 4.50	1.860	.395
	CC	4	0.50	0 to 2.50		
rs12899618	CC	46	1.0000	0 to 3		
	CT	14	1.0000	0 to 3	.163	.685
	TT	-	-	-		
rs12922394	GG	56	1.0000	0 to 3		
	GA	4	1.0000	0.25 to 1	1.007	.316
	AA	-	-	-		
rs1303	TT	28	2.0000	0 to 4.75		
	TC	27	1.0000	0 to 3	1.186	.553
	CC	5	1.0000	0 to 2.5		
rs1800450	CC	44	2.0000	0 to 2.75		
	CT	15	2.0000	0.75 to 3.25	5.100	.078
	TT	-	-	-		
rs5030737	GG	52	1.0000	0 to 3		
	GA	8	2.5000	1 to 5	3.148	.076
	AA	-	-	-		
rs7041	GG	22	1.0000	0 to 3.25		
	GT	29	1.0000	0 to 3	.889	.638
	TT	9	3.0000	0.5 to 4		

*Calculated using Kruskal-Wallis test

SNP- Single Nucleotide Polymorphism

IQR- Interquartile range

Table S 5-Associations between Borg scale (Dyspnoea) and genetic variants

SNP	Genotype	N	Median	IQR	Chi-Square	p-value*
rs10011792	CC	36	1	0 to 3.75		
	CT	22	0.5	0 to 2.25	1.126	.289
	TT	-	-	-		
rs1042522	CC	5	3.0000	0 to 4.5		
	CG	24	0.7500	0 to 3	.659	.719
	GG	30	1.0000	0 to 3		
rs1042713	CC	20	1.5000	0 to 3.75		
	TC	24	2.0000	0 to 4	5.268	.072
	TT	14	0.0000	0 to 1		
rs1042714	GG	30	0.0000	0 to 3		
	CG	23	3.0000	0 to 4	4.111	.128
	CC	5	0.0000	0 to 3		
rs1042717	CC	31	0.0000	0 to 3		
	CT†	24	3.0000	0.25 to 4	11.147	.004
	TT	4	0.0000	0 to 0		
rs10461985	GG	55	1.0000	0 to 3		
	GA	4	.0000	0 to 2.25	2.194	.13
	AA	-	-	-		
rs1051303	CC	8	3.0000	0.1250 to 4		
	CT	34	0.0000	0 to 3	2.351	.309
	TT	16	1.0000	0 to 3		
rs10516526	GG	50	1.0000	0 to 3		
	GA	9	1.0000	0 to 3.50	.084	.772
	AA	-	-	-		
rs1051740	AA	40	0.1250	0 to 3		
	AG	16	1.0000	0 to 3.75	.755	.685
	GG	2	2.5000	2 to -		
rs1059823	TT	14	3.0000	0 to 4		
	TC	25	0.0000	0 to 3	3.668	.160
	CC	20	0.7500	0 to 2.75		
rs10844154	TT	10	0.5000	0 to 3.25		
	TG	34	2.0000	0 to 4	3.666	.160
	GG	15	.0000	0 to 1		
rs11001819	GG	23	1.0000	0 to 3		
	GA	26	1.0000	0 to 3	.206	.902
	AA	10	1.7500	0 to 3.25		
rs11046966	AA	30	1.5000	0 to 3		
	AG	22	.0000	0 to 4	.513	.774
	GG	7	3.000	0 to 3		
rs11172113	AA	9	1.0000	.5 to 3		
	AG	22	.0000	0 to 3	1.379	.502
	GG	28	1.0000	0 to 4		
rs1129055	GG	36	1.5000	0 to 3		
	GA	19	0.0000	0 to 3	.524	.769
	AA	-	-	.		
rs1130866	TT	13	0.0000	0 to 3		
	TC	36	0.7500	0 to 3.75	.673	.714
	CC	10	3.0000	0 to 3		
rs1131620	CC	9	3.0000	0.25 to 4		
	CT	34	.0000	0 to 3	2.233	.327
	TT	16	1.0000	0 to 3		
rs1138272	CC#	52	.0000	0 to 3		
	CT	5	3.0000	2.5 to 4	4.924	.026
	TT	-	-	-		
rs1143634	GG	36	1.0000	0 to3		
	GA	18	.0000	0 to 3.25	1.057	.589
	AA	5	2.0000	0.5 to 4		
rs1155563	CC	2	2.5000	0 to -		
	CT	25	2.0000	0 to 4	3.586	.166
	TT	29	.0000	0 to 2.5		
rs11556218	TT	45	.0000	0 to 3		
	TG	14	1.5000	0 to 3	.904	.342
	GG	-	-	-		

rs11614913	CC	29	1.0000	0 to 3		
	CT	24	0.5000	0 to 4	.992	.609
	TT	6	2.5000	0 to 4		
rs11677877	TT	52	.7500	0 to 3		
	TG	6	2.0000	0 to 3.25	.176	.675
	GG	-	-	-		
rs11739136	GG	47	1.0000	0 to 3		
	GA	12	0.2500	0 to 2.75	.766	.381
	AA	-	-	-		
rs12477314	GG	38	1.0000	0 to 3		
	GA	19	1.0000	0 to 3	2.117	.347
	AA	2	.0000	0 to 3		
rs12504628	TT‡	30	.0000	0 to 1.50		
	TC	25	3.0000	0 to 4	9.092	.011
	CC	4	1.0000	0 to 2		
rs12899618	CC	45	1.0000	0 to 3		
	CT	14	1.0000	0 to 3.25	.000	.992
	TT	-	-	-		
rs12922394	GG	55	1.0000	0 to 3		
	GA	4	1.75000	0.1250 to 3.75	.260	.610
	AA	-	-	-		
rs1303	TT	28	1.0000	0 to 3		
	TC	26	1.0000	0 to 4	.684	.711
	CC	5	.0000	0 to 4		
rs1800450	CC	44	.7500	0 to 3		
	CT	14	2.0000	0 to 4	1.324	.516
	TT	-	-	-		
rs5030737	GG	51	1.0000	0 to 3		
	GA	8	3.0000	0 to 3.75	.749	.387
	AA	-	-	-		
rs7041	GG	22	.0000	0 to 3		
	GT	29	.2500	0 to 3	4.420	.110
	TT	9	3.0000	1 to 4.50		

*Calculated using Kruskal-Wallis test

†Significant different from CC and TT

#Significant different from CT

‡Significant different from TC

SNP- Single Nucleotide Polymorphism

IQR- Interquartile range

Table S 6-Associations between Borg scale (fatigue) and genetic variants

SNP	Genotype	N	Median	IQR	F.	p-value*
rs10011792	CC	36	0.2500	0 to 3.75		
	CT	22	.0000	0 to 2.25	.793	.373
	TT	-	-	-		
rs1042522	CC	5	.0000	0 yo 3		
	CG	24	.5000	0 to 3	.402	.818
	GG	30	.0000	0 to 3.25		
rs1042713	CC	20	1.2500	0 to 3.75		
	TC	24	.0000	0 to 4	2.632	.268
	TT	14	.0000	0 to 1		
rs1042714	GG†	30	.0000	0 to 1.25		
	GC	23	3.0000	0 to 4	6.784	.034
	CC	5	.0000	0 to 3		
rs1042717	CC	31	.0000	0 to 3		
	CT	24	2.5000	0 to 4	6.500	.039
	TT	4	.0000	0 to 0		
rs10461985	GG	55	.0000	0 to 3		
	GA	4	.0000	0 to 2.25	.691	.359
	AA	-	-	-		
rs1051303	CC	8	1.7500	0 to 4.50		
	CT	34	.0000	0 to 3	1.306	.520
	TT	16	.5000	0 to 3.75		
rs10516526	GG	50	.5000	0 to 3		
	GA	9	.0000	0 to 0.50	2.214	.137
	AA	-	-	-		
rs1051740	AA	40	0.0000	0 to 3		
	AG	16	1.2500	0 to 3.75	2.520	.284
	GG	2	2.5000	2 to -		
rs1059823	TT	14	0.0000	0 to 3		
	TC	25	0.0000	0 to 3.50	.173	.917
	CC	20	0.2500	0 to 2.75		
rs10844154	TT	10	1.0000	0 to 3.25		
	TG	34	.0000	0 to 3.25	1.009	.604
	GG	15	.0000	0 to 2		
rs11001819	GG	23	.0000	0 to 3		
	GA	26	.000	0 to 3	.970	.616
	AA	10	1.7500	0 to 4		
rs11046966	AA	30	.2500	0 to 3		
	AG	22	.2500	0 to 4.25	1.335	0.513
	GG	7	.0000	0 to 3		
rs11172113	AA	9	3.0000	0.5 to 4.5		
	AG	22	.0000	0 to 3	3.564	.168
	GG	28	.0000	0 to 3		
rs1129055	GG	36	1.5000	0 to 3		
	GA	19	.0000	0 to 3	1.865	.394
	AA	-	-	-		
rs1130866	TT	13	0.0000	0 to 3		
	TC	36	0.2500	0 to 3	.076	.963
	CC	10	1.5000	0 to 3		
rs1131620	CC	9	3.0000	0 to 4		
	CT	34	.0000	0 to 3	1.824	.402
	TT	16	.5000	0 to 3.75		
rs1138272	CC#	52	.0000	0 to 3		
	CT	5	3.0000	1.75 to 4	5.443	.020
	TT	-	-	-		
rs1143634	GG	36	.0000	0 to 3		
	GA	18	.7500	0 to 3	.306	.858
	AA	5	.0000	0 to 3		
rs1155563	CC	2	.5000	0 to -		
	CT	25	.0000	0 to 3	.100	.971
	TT	29	.0000	0 to 3		
rs11556218	TT	45	0.5000	0 to 3		
	TG	14	.0000	0 to 3	.725	.395
	GG	-	-	-		

rs11614913	CC	29	.0000	0 to 3		
	CT	24	.2500	0 to 3	.724	.696
	TT	6	1.5000	0 to 4		
rs11677877	TT	52	.0000	0 to 3		
	TG	6	3.0000	0 to 4.75	1.465	.226
	GG	-	-	-		
rs11739136	GG	47	.0000	0 to 3		
	GA	12	.0000	0 to 3	.121	.728
	AA	-	-	-		
rs12477314	GG	38	.0000	0 to 3		
	GA	19	2.0000	0 to 4	4.075	.130
	AA	2	.0000	.00000		
rs12504628	TT	30	.0000	0 to 3		
	TC	25	.5000	0 to 3.50	2.562	.278
	CC	4	2.5000	0.50 to 3.75		
rs12899618	CC	45	.0000	0 to 3		
	CT	14	.0000	0 to 2.25	.775	.379
	TT	-	-	-		
rs12922394	GG	55	.0000	0 to 3		
	GA	4	2.2500	.1250 to 8.50	1.630	.202
	AA	-	-	-		
rs1303	TT	28	.0000	0 to 3		
	TC	26	1.0000	0 to 4	2.455	.293
	CC	5	.0000	0 to 6.5		
rs1800450	CC	44	.0000	0 to 3		
	CT	14	.2500	0 to 3.25	.942	.624
	TT	-	-	-		
rs5030737	GG	51	.0000	0 to 3		
	GA	8	1.5000	0 to 3	.033	.857
	AA	-	-	-		
rs7041	GG	22	.25000	0 to 3.25		
	GT	28	.0000	0 to 3	2.608	.271
	TT	9	3.0000	0 to 4		

*Calculated using Kruskal-Wallis test

†Significant different from GC

#Significant different from CT

SNP- Single Nucleotide Polymorphism

IQR- Interquartile range

Table S 7-Associations between Chronic Obstructive Pulmonary Disease Assessment Test and genetic variants

SNP	Genotype	N	Median	IQR	Chi-Square	p-value*
rs10011792	CC	37	21	14.25 to 25.75		
	CT	22	19	11.75 to 25.50	.285	.594
	TT	-	-	-		
rs1042522	CC	5	17.0000	10 to 27.50		
	CG	25	17.5000	12.25 to 26.25	.717	.699
	GG	5	21.0000	15.50 to 25		
rs1042713	CC	21	21.5000	15.25 to 25.75		
	TC	24	20.5000	11.75 to 26.75	.801	.670
	TT	14	16.5000	10.25 to 24.25		
rs1042714	GG	30	16.5000	10.75 to 24.25		
	CG	24	21.0000	17 to 27	3.250	.197
	CC	5	25.0000	19 to 27		
rs1042717	CC	31	21.0000	15 to 25		
	CT	25	21.0000	13.25 to 26.75	1.060	.589
	TT	4	14.0000	13 to 21.75		
rs10461985	GG†	56	21.0000	14 to 26		
	GA	4	12.0000	7.50 to 16.50	3.893	.048
	AA	-	-	-		
rS1051303	CC	8	18.5000	11.75 to 26.50		
	CT	35	17.5000	13 to 24.25	2.209	.331
	TT	16	23.5000	17.25 to 26.75		
rs10516526	GG	50	21.0000	14.75 to 25		
	GA	10	18.0000	12 to 26.50	.663	.416
	AA	-	-	-		
rs1051740	AA	41	21.0000	14.25 to 25.75		
	AG	16	17.5000	13 to 23.75	2.037	.361
	GG	2	26.0000	23 to -		
rs1059823	TT	14	19.0000	14.75 to 25.		
	TC	26	21.0000	13 to 23.50	.947	.623
	CC	20	20.5000	13 to 27.50		
rs10844154	TT	10	21.0000	9 to 26.75		
	TG	35	17.0000	14.75 to 24.25	.732	.694
	GG	15	23.0000	14 to 26		
rs11001819	GG	23	17.0000	11 to 25		
	GA	27	21.0000	16.50 to 26.25	.868	.648
	AA	10	17.5000	12.50 to 27.25		
rs11046966	AA	30	23.0000	15.50 to 27		
	AG	23	17.0000	14.40 to 24.25	3.716	.156
	GG	7	14.0000	10 to 22		
rs11172113	AA#	9	23.0000	20.50 to 28.50		
	AG	22	15.0000	8.75 to 21.25	9.668	.008
	GG	29	22.0000	15.25 to 26.75		
rs1129055	GG	37	21.0000	15.25 to 24.75		
	GA	19	17.0000	10 to 27	.393	.822
	AA	-	-	-		
rs1130866	TT	13	23.0000	15.50 to 27		
	TC	37	17.5000	14 to 25.50	1.558	.459
	CC	10	20.0000	10.50 to 25.25		
rs1131620	CC	9	19.5556	12.50 to 26		
	CT	35	18.1429	13 to 24.25	2.267	.322
	TT	16	21.4375	17.25 to 26.25		

rs1138272	CC	53	20.5000	13.25 to 25		
	CT	5	21.0000	19.50 to 26.50	.889	.346
	TT	-	-	-		
rs1143634	GG	36	18.0000	13 to 23.75		
	GA	19	25.0000	15.25 to 29	.1.971	.0.373
	AA	5	20.0000	16 to 22.50		
rs1155563	CC	2	23.0000	17 to -		
	CT	25	21.0000	§3.50 to 26	.129	.720
	TT	30	20.0000	12.50 to 24.50		
rs11556218	TT	46	21.0000	13.50 to 25		
	TG	14	20.0000	14.75 to 27	.063	.803
	GG	-	-	-		
rs11614913	CC	30	21.0000	13 to 26		
	CT	24	18.5000	14.25 to 25.75	.002	.999
	TT	6	19.0000	14.25 to 26		
rs11677877	TT	53	20.5000	13 to 25		
	TG	6	25.0000	17 to 29.25	2.542	.111
	GG	-	-	-		
rs11739136	GG	47	20.0000	14 to 26		
	GA	13	22.5000	12.25 to 25	.029	.864
	AA	-	-	-		
rs12477314	GG	39	20.5000	13 to 26		
	GA	19	21.0000	16 to 25	1.150	.563
	AA	2	14.0000	9 to -		
rs12504628	TT	31	17.0000	12.75 to 24		
	TC	25	22.0000	15 to 26.50	3.798	.150
	CC	4	25.000	17.25 to 29		
rs12899618	CC	46	21.0000	13.75 to 25		
	CT	14	17.0000	10.75 to 27.50	.344	.558
	TT	-	-	-		
rs12922394	GG	56	20.0000	14 to 25		
	GA	4	23.0000	13.50 to 31	.528	.467
	AA	-	-	-		
rs1303	TT	28	17.5000	13 to 23.75		
	TC	27	21.0000	15.75 to 26.25	1.491	.475
	CC	5	8.0000	6.50 to 29.50		
rs1800450	CC	44	21.0000	14 to 26		
	CT	15	17.5000	12.75 to 22	1.864	.394
	TT	-	-	-		
rs5030737	GG	52	20.0000	14 to 26		
	GA	8	22.0000	14 to 24.75	.034	.853
	AA	-	-	-		
rs7041	GG	22	19.0000	13 to 24.25		
	GT	29	20.0000	13.25 to 26	1.014	.602
	TT	9	21.0000	17 to 27		

*Calculated using Kruskal-Wallis test

†Significant different from GA

#Significant different from AG

SNP- Single Nucleotide Polymorphism

IQR- Interquartile range

Table S 8-Associations between Hospital Anxiety and Depression scale (Anxiety score) and genetic variants

SNP	Genotype	N	Median	IQR	Chi-Square	p-value*
rs10011792	CC	37	8	5 to 13		
	CT	22	7	5.50 to 9.25	.645	.422
	TT	-	-	-		
rs1042522	CC	5	8.0000	4.50 to 10.50		
	CG	25	7.5000	5.25 to 9.75	.280	.870
	GG	30	7.0000	5 to 13		
rs1042713	CC	21	8.0000	6 to 10.50		
	TC	24	7.0000	5 to 10.75	1.784	.410
	TT	14	6.0000	2.75 to 10.50		
rs1042714	GG	30	8.0000	5 to 11		
	CG	24	6.0000	5 to 11	.497	.780
	CC	5	8.0000	4 to 14.50		
rs1042717	CC	31	6.0000	3 to 11		
	CT	25	8.0000	6 to 13	3.696	.158
	TT	4	9.0000	8.25 to 10.50		
rs10461985	GG	56	8.0000	5 to 11		
	GA	4	7.0000	1.75 to 7.75	.622	.430
	AA	-	-	-		
rS1051303	CC†	8	11.0000	7.50 to 13.75		
	CT	35	6.0000	4 to 9.25	6.199	.045
	TT	16	8.0000	6 to 13		
rs10516526	GG	50	7.0000	5 to 11		
	GA	10	8.0000	6.50 to 12	.219	.640
	AA	-	-	-		
rs1051740	AA	41	6.0000	4.25 to 10		
	AG	16	8.0000	6.25 to 12.50	3.147	.207
	GG	2	6.0000	6 to -		
rS1059823	TT	14	10.0000	5.75 to 13		
	TC	26	7.0000	6 to 9	1.757	.415
	CC	20	6.5000	3.50 to 10.75		
rs10844154	TT	10	7.0000	3.59629		
	TG	35	8.0000	4.95831	.536	.765
	GG	15	7.0000	4.46681		
rs11001819	GG	10	7.0000	6 to 10		
	GA	35	7.5000	5 to 11	.538	.764
	AA	15	7.0000	4.50 to 14		
rs11046966	AA	30	8.0000	6 to 10.25		
	AG	23	6.0000	4.75 to 13	.701	.704
	GG	7	9.0000	5 to 11		
rs11172113	AA	9	6.0000	5.50 to 12		
	AG	22	6.5000	4.75 to 9	1.117	.572
	GG	29	8.0000	6 to 13		
rs1129055	GG	37	8.0000	6 to 10.75		
	GA	19	7.0000	4 to 13	.678	.713
	AA	-	-	-		
rs1130866	TT	13	9.0000	5 to 12		
	TC	37	7.0000	5.25 to 10.75	.512	.774
	CC	10	7.5000	4.75 to 11.50		
rS1131620	CC‡	9	13.0000	8 to 13.50		
	CT	35	6.0000	4 to 9.25	7.429	.024
	TT	16	8.0000	6 to 13		
rs1138272	CC	53	7.6038	4.72041		
	CT	5	9.2000	3.03315	.545	.463
	TT	-	-	-		
rs1143634	GG	36	7.0000	5 to 10.75		
	GA	19	8.0000	5.50 to 11.50	1.253	.534
	AA	5	6.0000	2.50 to 9.50		
rs1155563	CC	2	3.5000	1 to -		
	CT	25	8.0000	5 to 12	2.617	.270
	TT	30	7.0000	5 to 9		
rs11556218	TT	46	7.0000	5 to 11		
	TG	14	7.5000	5.50 to 11.50	.031	.861
	GG	-	-	-		

rs11614913	CC	30	7.0000	5 to 10.50		
	CT	24	8.0000	5.25 to 12.50	.868	.648
	TT	6	6.5000	5 to 14.25		
rs11677877	TT	53	7.0000	5 to 11		
	TG	6	7.0000	4.25 to 9.75	.057	.811
	GG	-	-	-		
rs11739136	GG	47	8.0000	5 to 11		
	GA	13	6.0000	4.25 to 13	.469	.493
	AA	-	-	-		
rs12477314	GG	39	8.0000	5 to 11.50		
	GA	19	7.0000	6 to 9	1.229	.541
	AA	2	4.0000	1 to -		
rs12504628	TT	31	7.0000	4.50 to 9.25		
	TC	25	8.0000	6 to 13	3.183	.204
	CC	4	6.0000	4.50 to 9		
rs12899618	CC	46	7.5000	5 to 13		
	CT	14	6.000	2.75 to 9	1.532	.216
	TT	-	-	-		
rs12922394	GG	56	7.0000	5 to 11		
	GA	4	11.0000	5.75 to 14.75	1.418	.234
	AA	-	-	-		
rs1303	TT	28	8.0000	6 to 10.75		
	TC	27	6.0000	5 to 11.50	.236	.790
	CC	5	4.0000	2.50 to 12.50		
rs1800450	CC#	44	8.0000	6 to 12.50		
	CT	15	6.0000	2 to 6.50	8.093	.017
	TT	-	-	-		
rs5030737	GG	52	7.0000	5 to 11		
	GA	8	9.5000	6.75 to 12.50	2.720	.099
	AA	-	-	-		
rs7041	GG	22	7.5000	5 to 10		
	GT	29	7.5000	5 to 11	.014	.993
	TT	9	6.0000	3.50 to 13		

*Calculated using Kruskal-Wallis test

†Significant different from CT

#Significant different from CT

‡Significant different from CT

SNP- Single Nucleotide Polymorphism

IQR- Interquartile range

Table S 9-Associations between Hospital Anxiety and Depression scale (Depression score) and genetic variants

SNP	Genotype	N	Median	IQR	Chi-Square	p-value*
rs10011792	CC	37	8.0000	3.79070		
	CT	22	8.0000	4.35766	.897	.344
	TT	-	-	-		
rs1042522	CC	5	7.0000	4.5 to 10		
	CG	25	6.5000	3 to 9	1.530	.465
	GG	30	8.0000	4.75 to 10.50		
rs1042713	CC	21	8.0000	5 to 9		
	TC	24	7.5000	3 to 10.75	.088	.957
	TT	14	8.0000	4 to 9		
rs1042714	GG	30	8.0000	4 to 9		
	CG	24	8.0000	3 to 14	.075	.963
	CC	5	6.0000	4 to 10		
rs1042717	CC	31	8.0000	3 to 9		
	CT	25	7.5000	5 to 10.50	.356	.837
	TT	4	9.0000	4 to 9		
rs10461985	GG	56	8.0000	4 to 9		
	GA	4	3.0000	2.50 to 9	2.050	.152
	AA	-	-	-		
rS1051303	CC	8	8.0000	3.75 to 9		
	TC	35	8.0000	3 to 9	.428	.807
	TT	16	7.5000	4.25 to 12		
rS10516526	GG	50	8.0000	3.75 to 9.25		
	GA	10	7.0000	3.50 to 8	.687	.407
	AA	-	-	-		
rs1051740	AA	41	8.0000	4.25 to 10		
	AG	16	8.0000	6.25 to 12.50	.166	.920
	GG	2	8.0000	6 to -		
rs1059823	TT	14	6.0000	2.75 to 8		
	TC	26	8.0000	4 to 10.50	2.641	.267
	CC	20	8.0000	5.25 to 9		
rs10844154	TT	10	8.5000	2.75 to 9.75		
	TG	35	8.0000	4 to 11	1.243	.537
	GG	15	6.0000	4 to 8		
rs11001819	GG	23	6.0000	3 to 11		
	GA	27	8.0000	3.75 to 9	.793	.673
	AA	10	8.0000	4.50 to 9.75		
rs11046966	AA	30	8.0000	3 to 9		
	AG	23	8.0000	4 to 11.25	1.987	.370
	GG	7	5.0000	3 to 9		
rs11172113	AA	9	9.0000	4 to 14		
	AG	22	7.5000	3 to 8.25	2.454	.293
	GG	29	8.0000	4.25 to 11.75		
rs1129055	GG	37	8.0000	4.25 to 9		
	GA	19	6.0000	3 to 9	2.009	.366
	AA	-	-	-		
rs1130866	TT	13	8.0000	4 to 9.50		
	TC	37	8.0000	3.25 to 9	.058	.972
	CC	10	7.5000	3.75 to 9.75		
rs1131620	CC	9	8.0000	4.50 to 9		
	CT	35	8.0000	3 to 9	.350	.839
	TT	16	7.5000	4.25 to 12		
rs1138272	CC	53	7.0000	5 to 11		
	CT	5	8.0000	7 to 12	.1.059	.303
	TT	-	-	-		
rs1143634	GG	36	7.0000	3.25 to 9		
	GA	19	8.0000	4 to 11	1.959	.376
	AA	5	6.0000	3 to 10.50		
rs1155563	CC	2	7.5000	6 to -		
	CT	25	8.0000	3 to 10.50	.145	.930
	TT	30	8.0000	3.5 to 9		
rs11556218	TT	46	8.0000	3.50 to 9		
	TG	14	7.0000	3.75 to 11.25	.011	.916
	GG	-	-	-		

rs11614913	CC	30	7.0000	3 to 10		
	CT	24	8.0000	5.25 to 9	2.584	.275
	TT	6	5.5000	2.75 to 10.50		
rs11677877	TT	53	8.0000	3 to 9		
	TG	6	9.5000	6.50 to 12	1.757	.185
	GG	-	-	-		
rs11739136	GG	47	8.0000	3 to 9		
	GA	13	8.0000	4 to 9	.094	.759
	AA	-	-	-		
rs12477314	GG	39	7.5000	3 to 9.50		
	GA	19	8.0000	5 to 9	.096	.908
	AA	2	8.5000	3 to -		
rs12504628	TT	31	7.0000	3 to 9		
	TC	25	8.0000	5 to 11	.890	.641
	CC	4	6.5000	3.25 to 10.50		
rs12899618	CC	46	8.0000	3 to 9.25		
	CT	14	7.0000	4.75 to 9	.020	.888
	TT	-	-	-		
rs12922394	GG	56	8.0000	3 to 9		
	GA	4	8.0000	5.75 to 11	.356	.551
	AA	-	-	-		
rs1303	TT	28	7.5000	3.50 to 9		
	TC	27	8.0000	3 to 9	.338	.844
	CC	5	8.0000	4 to 12		
rs1800450	CC	44	7.5000	4 to 9		
	CT	15	8.0000	3 to 10.50	1.674	.433
	TT	-	-	.		
rs5030737	GG	52	7.0000	3 to 9		
	GA	8	8.5000	6.50 to 9	1.346	.246
	AA	-	-	-		
rs7041	GG	22	8.0000	3 to 9.50		
	GT	29	7.5000	4 to 9	.805	.669
	TT	9	9.0000	6 to 10.50		

*Calculated using Kruskal-Wallis test

SNP- Single Nucleotide Polymorphism

IQR- Interquartile range

Table S 10-Associations between St. George Respiratory Questionnaire (Symptoms score) and genetic variants

SNP	Genotype	N	Mean	Std. Deviation	F.	p-value*
rs10011792	CC	34	52.5979	20.80043		
	CT	22	54.5625	19.84468	.123	.727
	TT	-	-	-		
rs1042522	CC	5	55.0720	29.48990		
	CG	23	51.3709	21.33971	.132	.877
	GG	29	53.9994	18.27664		
rs1042713	CC	18	50.6439	15.89985		
	TC	24	56.8910	21.93412	.625	.539
	TT	14	50.8379	22.72766		
rs1042714	GG	29	55.4126	21.92192		
	CG	23	52.4630	19.08857	.610	.547
	CC	4	43.7725	14.36144		
rs1042717	CC	30	49.8437	20.83650		
	CT	24	57.8843	19.70921	1.247	.296
	TT	3	46.1133	14.53889		
rs10461985	GG	53	53.9788	19.41973		
	GA	4	40.5000	29.86188	1.668	.202
	AA	-	-	-		
rs1051303	CC	7	46.5500	27.09478		
	TC	33	53.7813	20.47939	.482	.620
	TT	16	55.5044	17.06751		
rs10516526	GG	48	52.6736	20.39925		
	GA	9	54.9489	20.50653	.094	.760
	AA	-	-	-		
rs1051740	AA	39	52.4969	20.35840		
	AG	15	53.2249	20.80802	.829	.442
	GG	2	71.4750	10.11870		
rs1059823	TT	13	56.2123	19.53700		
	TC	24	50.1577	18.78003	.440	.646
	CC	20	54.4165	22.82791		
rs10844154	TT	10	50.4914	24.79301		
	TG	32	54.1163	20.45977	.127	.881
	GG	15	52.4160	17.61633		
rs11001819	GG	23	53.5750	19.54653		
	GA	25	53.9952	21.47041	.211	.810
	AA	9	48.9744	20.35422		
rs11046966	AA	29	54.5288	18.73832		
	AG	22	52.0214	23.49962	.192	.826
	GG	6	49.5117	16.56080		
rs11172113	AA †	9	66.9278	18.39889		
	AG	20	42.5840	19.78069	5.996	.004
	GG	28	56.0301	17.89509		
rs1129055	GG	34	55.3632	19.22733		
	GA	19	47.7986	18.72939	.984	.380
	AA	-	-	-		
rs1130866	TT	13	57.9423	23.55151		
	TC	35	51.0106	19.20872	.554	.578
	CC	9	53.8060	20.30511		
rs1131620	CC	8	45.0025	25.46389		
	CT	33	53.7813	20.47939	.764	.471
	TT	16	55.5044	17.06751		

rs1138272	CC	50	52.3809	20.41150		
	CT	5	66.3880	16.14998	2.203	.144
	TT	-	-	-		
rs1143634	GG	35	52.9329	20.53516		
	GA	17	52.2955	22.53929	.072	.931
	AA	5	56.2400	10.42320		
rs1155563	CC	2	53.1300	26.43165		
	CT	24	56.7996	19.82614	.585	.561
	TT	28	50.5430	21.34634		
rs11556218	TT	44	51.7340	19.86672		
	TG	13	57.4292	21.72641	.791	.378
	GG	-	-	-		
rs11614913	CC	29	47.9128	17.30064		
	CT	23	60.2597	21.79640	2.611	.083
	TT	5	49.4860	22.93741		
rs11677877	TT	50	53.1941	19.94553		
	TG	6	54.8333	24.86572	.034	.854
	GG	-	-	-		
rs11739136	GG	45	53.3390	20.86103		
	GA	12	51.8850	18.57023	.048	.827
	AA	-	-	-		
rs12477314	GG	37	54.7838	19.41736		
	GA	18	50.7058	20.09206	.568	.570
	AA	2	41.5850	45.30433		
rs12504628	TT	29	52.0157	19.80469		
	TC	24	54.3704	21.59501	.088	.916
	CC	4	52.3825	19.82074		
rs12899618	CC	44	54.3651	19.28426		
	CT	13	48.5238	23.50194	.833	.366
	TT	-	-	-		
rs12922394	GG	53	52.6725	20.18728		
	GA	4	57.8075	23.60813	.236	.629
	AA	-	-	-		
rs1303	TT	28	52.9012	21.22131		
	TC	24	54.0875	15.45051	.143	.867
	CC	5	48.7080	36.02415		
rs1800450	CC	43	53.4133	20.62229		
	CT	13	51.3023	20.44694	.098	.907
	TT	-	-	-		
rs5030737	GG	49	51.4292	20.40153		
	GA	8	62.8555	17.25806	2.238	.140
	AA	-	-	-		
rs7041	GG	21	52.5550	21.19238		
	GT	27	54.0374	22.15838	.076	.927
	TT	9	51.1344	11.98335		

*Calculated using One-way ANOVA test

†Significant different from AG

SNP- Single Nucleotide Polymorphism

Table S 11-Associations between St. George Respiratory Questionnaire (Activity score) and genetic variants

SNP	Genotype	N	Mean	Std. Deviation	F.	p-value*
rs10011792	CC	34	70.2118	19.37017		
	CT	22	66.7077	23.16256	.374	.543
	TT	-	-	-		
rs1042522	CC	5	65.3880	21.04737		
	CG	23	68.5491	21.03862	.101	.904
	GG	29	69.8234	20.90495		
rs1042713	. CC†	18	79.0311	15.91436		
	TC	24	63.2325	22.08733	3.526	.036
	TT	14	65.3307	20.54955		
rs1042714	GG	29	65.8476	20.97003		
	CG	23	70.5243	21.55962	1.035	.362
	CC	4	80.7825	11.22677		
rs1042717	CC	30	64.7257	20.09562		
	CT	24	75.2208	20.95140	2.067	.136
	TT	3	60.4600	12.89910		
rs10461985	GG	53	70.0085	20.04660		
	GA	4	54.5000	26.10092	2.145	.149
	AA	-	-	-		
rs1051303	CC	7	75.7657	20.64956		
	TC	33	63.5048	19.50543	2.817	.069
	TT	16	76.7969	21.27762		
rs10516526	GG	48	69.9683	21.31517		
	GA	9	63.3300	16.38430	.782	.380
	AA	-	-	-		
rs1051740	AA	39	68.2987	21.72775		
	AG	15	68.8500	19.98075	.253	.777
	GG	2	79.1850	7.61554		
rs1059823	TT	13	69.0154	24.09696		
	TC	24	69.7317	20.86936	.042	.959
	CC	20	67.8845	18.93595		
rs10844154	TT	10	66.5920	28.35055		
	TG	32	69.8572	20.32893	.097	.908
	GG	15	68.4733	16.26797		
rs11001819	GG	23	67.8448	22.38554		
	GA	25	68.8240	19.07342	.124	.884
	AA	9	71.9356	22.27987		
rs11046966	AA	29	70.9169	20.22739		
	AG	22	67.5673	21.28586	.330	.720
	GG	6	64.2300	22.70032		
rs11172113	AA	9	75.9400	18.66911		
	AG#	20	58.7865	22.24360	4.175	.021
	GG	28	73.9021	17.69496		
rs1129055	GG	34	71.7609	19.86770		
	GA	19	65.9058	21.03647	.978	.383
	AA	-	-	-		
rs1130866	TT	13	67.0238	20.88121		
	TC	35	70.1123	20.78886	.147	.864
	CC	9	67.0233	21.73759		
rs1131620	CC	8	75.5050	19.13199		
	CT	33	63.5048	19.50543	2.894	.064
	TT	16	76.7969	21.27762		
rs1138272	CC‡	50	66.3212	20.23950		
	CT	5	89.2400	13.98030	6.068	.017
	TT	-	-	-		
rs1143634	GG	35	65.5640	21.28671		
	GA	17	73.3806	20.42301	1.280	.286
	AA	5	77.2480	13.00318		
rs1155563	CC	2	82.7800	13.76030		
	CT	24	64.5321	25.44051	.974	.384
	TT	28	70.0450	16.06988		
rs11556218	TT	44	70.1861	20.14395		
	TG	13	64.6354	22.50498	.723	.399
	GG	-	-	-		

rs11614913	CC	29	68.2883	17.47859		
	CT	23	68.4187	23.17694	.223	.800
	TT	5	74.8920	28.58279		
rs11677877	TT	50	68.0170	21.45566		
	TG	6	75.6533	13.73316	.718	.401
	GG	-	-	-		
rs11739136	GG	45	69.3709	20.03877		
	GA	12	67.2300	23.59438	.100	.753
	AA	-	-	-		
rs12477314	GG	37	70.4795	21.16130		
	GA	18	68.7511	18.98916	1.922	.156
	AA	2	41.5950	.85560		
rs12504628	TT	29	64.9393	20.82707		
	TC	24	72.7921	19.87723	1.116	.335
	CC	4	74.5500	23.49718		
rs12899618	CC	44	68.9209	20.40090		
	CT	13	68.9177	22.24653	.000	1.000
	TT	-	-	-		
rs12922394	GG	53	68.4591	21.16911		
	GA	4	75.0300	11.13478	.373	.544
	AA	-	-	-		
rs1303	TT	28	67.3643	20.08433		
	TC	24	73.4629	19.33415	1.711	.190
	CC	5	55.8280	27.04807		
rs1800450	CC	43	70.2458	19.66751		
	CT	13	64.1600	24.48699	.454	.638
	TT	-	-	-		
rs5030737	GG	49	67.7618	21.06921		
	GA	8	76.0150	17.15133	1.103	.298
	AA	-	-	-		
rs7041	GG	21	71.3471	14.27988		
	GT	27	63.2856	23.00992	2.633	.081
	TT	9	80.1611	22.00300		

*Calculated using One-way ANOVA

†Significant different from CT

#Significant different from GG

‡Significant different from CT

SNP- Single Nucleotide Polymorphism

Table S 12-Associations between St. George Respiratory Questionnaire (Impact Score) and genetic variants

SNP	Genotype	N	Mean	Std. Deviation	F.	p-value*
rs10011792	CC	34	45.2656	18.62708		
	CT	22	42.3900	21.09715	.287	.594
	TT	-	-	-		
rs1042522	CC	5	38.3660	15.39285		
	CG	23	44.7643	17.00941	.241	.786
	GG	29	44.7200	21.88894		
rs1042713	CC	18	50.0578	18.76757		
	TC	24	43.1163	19.95268	1.525	.227
	TT	14	38.2700	18.86203		
rs1042714	GG	29	20.98933	3.89762		
	CG	23	18.79014	3.91802	.735	.484
	CC	4	8.94930	4.47465		
rs1042717	CC	30	39.7777	15.92838		
	CT	24	51.3600	21.72833	3.431	.040
	TT	3	30.7733	13.02492		
rs10461985	GG	53	44.8728	19.55990		
	GA	4	35.0075	14.79869	.969	.329
	AA	-	-	-		
rS1051303	CC	7	52.5300	23.80603		
	TC	33	40.6036	18.48712	1.491	.234
	TT	16	47.7487	19.07243		
rs10516526	GG	48	45.3219	19.19479		
	GA	9	38.0933	20.00299	1.062	.307
	AA	-	-	-		
rs1051740	AA	39	42.6892	20.05267		
	AG	15	46.1080	18.81363	.649	.526
	GG	2	57.5550	13.10269		
rs1059823	TT	13	46.3362	23.35305		
	TC	24	43.7308	19.49193	.104	.902
	CC	20	43.3190	17.09208		
rs10844154	TT	10	37.8120	18.49690		
	TG	32	46.6250	19.32087	.812	.449
	GG	15	43.2113	20.09151		
rs11001819	GG	23	40.2809	18.57520		
	GA	25	48.1748	17.88285	1.018	.368
	AA	9	43.0511	24.71737		
rs11046966	AA	29	47.1769	19.04418		
	AG	22	43.5845	19.33900	1.608	.210
	GG	6	31.8833	18.59641		
rs11172113	AA	9	52.7144	17.03475		
	AG	20	35.6425	20.13032	3.547	.036
	GG	28	47.5361	17.70555		
rs1129055	GG	34	46.6491	18.64518		
	GA	19	39.8668	20.52576	.745	.479
	AA	-	-	-		
rs1130866	TT	13	45.8315	18.91883		
	TC	35	44.0257	19.33429	.084	.920
	CC	9	42.3978	21.92031		
rs1131620	CC	8	51.7988	22.13694		
	CT	33	40.6036	18.48712	1.485	.236
	TT	16	47.7487	19.07243		
rs1138272	CC†	50	42.2744	18.97115		
	CT	5	60.6500	20.15434	4.224	.045
	TT	-	-	-		
rs1143634	GG	35	40.8691	18.98948		
	GA	17	47.7900	19.76881	1.644	.203
	AA	5	55.0880	17.32199		
rs1155563	CC	2	55.4050	8.40750		
	CT	24	44.8196	22.71234	.520	.597
	TT	28	41.7521	17.38502		
rs11556218	TT	44	44.4593	19.36247		
	TG	13	43.2369	19.95354	.039	.843
	GG	-	-	-		

rs11614913	CC	29	43.3938	18.88644		
	CT	23	43.2970	18.23409	.537	.587
	TT	5	52.8080	28.29946		
rs11677877	TT	50	43.2586	19.42261		
	TG	6	51.4467	20.31481	.944	.336
	GG	-	-	-		
rs11739136	GG	45	43.4233	16.27253		
	GA	12	47.0200	28.82566	.324	.571
	AA	-	-	-		
rs12477314	GG	37	44.4222	20.29817		
	GA	18	44.8211	18.48005	.286	.753
	AA	2	33.9450	7.50240		
rs12504628	TT#	29	37.0803	16.61336		
	TC	24	50.2713	18.72004	4.957	.011
	CC	4	59.1125	25.04178		
rS12899618	CC	44	44.4118	18.58080		
	CT	13	43.3977	22.46800	.027	.870
	TT	-	-	-		
rs12922394	GG	53	44.1387	19.32656		
	GA	4	44.7350	22.28217	.003	.953
	AA	-	-	-		
rs1303	TT	28	43.0389	19.42410		
	TC	24	47.0058	17.39732	.641	.531
	CC	5	37.0120	28.63704		
rs1800450	CC	43	44.9816	19.06801		
	CT	13	40.9985	21.28339	.270	.764
	TT	-	-	-		
rs5030737	GG	49	43.5278	19.45506		
	GA	8	48.1788	19.26104	.394	.533
	AA	-	-	-		
rs7041	GG	21	42.3200	15.50570		
	GT	27	41.7267	21.02765	2.038	.140
	TT	9	55.8833	19.81484		

*Calculated using One-way ANOVA

†Significant different from CT

#Significant different from TC

SNP- Single Nucleotide Polymorphism

Table S 13-Associations between St. George Respiratory Questionnaire (Total Score) and genetic variants

SNP	Genotype	N	Mean	Std. Deviation	F.	p-value*
rs10011792	CC	34	53.9150	16.79465		
	CT	22	51.7823	19.66819	.188	.666
	TT	-	-	-		
rs1042522	CC	5	49.2640	18.69689		
	CG	23	53.0039	16.97328	.136	.873
	GG	29	53.7972	18.58202		
rs1042713	CC	18	58.8878	16.07853		
	TC	24	51.3442	18.26215	1.544	0.223
	TT	14	48.5771	18.52970		
rs1042714	GG	29	50.9462	19.53534		
	CG	23	55.2074	16.81341	.426	.655
	CC	4	56.2775	10.17319		
rs1042717	CC	30	48.8740	15.44293		
	CT	24	59.7158	19.01201	3.393	.041
	TT	3	42.0433	12.44685		
rs10461985	GG	53	53.9223	17.42378		
	GA	4	41.9125	19.74256	1.740	.193
	AA	-	-	-		
rs1051303	CC	7	58.4929	22.24824		
	TC	33	49.6070	16.93087	1.555	.221
	TT	16	57.8650	17.08802		
rs10516526	GG	48	53.9335	17.77584		
	GA	9	48.5244	17.39081	.706	.404
	AA	-	-	-		
rs1051740	AA	39	51.9641	18.29773		
	AG	15	54.2107	17.39855	.646	.528
	GG	2	66.2800	10.74802		
rs1059823	TT	13	54.7692	21.56489		
	TC	24	52.5471	17.19216	.075	.928
	CC	20	52.6200	16.32813		
rs10844154	TT	10	48.6650	20.86285		
	TG	32	54.7756	17.43008	.461	.633
	GG	15	52.4040	16.61153		
rs11001819	GG	23	50.8313	18.44772		
	GA	25	55.2424	16.03978	.366	.695
	AA	9	52.8167	21.20415		
rs11046966	AA	29	55.6352	17.20112		
	AG	22	52.0941	18.39924	1.074	.349
	GG	6	44.3400	16.90871		
rs11172113	AA	9	62.1211	14.38634		
	AG#	20	43.5285	18.32917	5.566	.006
	GG	28	56.9954	15.47263		
rs1129055	GG	34	55.6129	16.65029		
	GA	19	49.0768	18.47931	.873	.423
	AA	-	-	-		
rs1130866	TT	13	53.8685	17.23610		
	TC	35	53.1357	17.70014	.038	.962
	CC	9	51.7211	20.15723		
rs1131620	CC	8	57.8325	20.68237		
	CT	33	49.6070	16.93087	1.542	.223
	TT	16	57.8650	17.08802		
rs1138272	CC	50	51.1408	17.48064		
	CT†	5	70.3940	13.48814	5.688	.021
	TT	-	-	-		
rs1143634	GG	35	50.2094	17.21339		
	GA	17	56.3082	19.00697	1.431	.248
	AA	5	62.1920	13.75839		
rs1155563	CC	2	63.6900	4.76590		
	CT	24	52.5529	21.17344	.401	.672
	TT	28	51.7786	15.50849		
rs11556218	TT	44	53.3700	17.41088		
	TG	13	52.0962	19.23777	.051	.822
	GG	-	-	-		

rs11614913	CC	29	51.6714	16.16983		
	CT	23	53.5513	18.14183	.379	.686
	TT	5	59.0760	25.92389		
rs11677877	TT	50	52.3184	17.97764		
	TG	6	59.4000	16.65586	.842	.363
	GG	-	-	-		
rs11739136	GG	45	52.8447	15.77574		
	GA	12	53.9600	24.36484	.037	.848
	AA	-	-	-		
rs12477314	GG	37	54.0508	18.66852		
	GA	18	52.8461	15.86391	.859	.429
	AA	2	37.2100	11.31371		
rs12504628	TT	29	47.9672	16.38147		
	TC	24	57.6562	17.19982	2.774	.071
	CC	4	62.6825	22.69210		
rs12899618	CC	44	53.4361	16.85865		
	CT	13	51.8723	20.90974	.077	.782
	TT	-	-	-		
rs12922394	GG	53	52.8525	17.85170		
	GA	4	56.0875	17.14547	.123	.728
	AA	-	-	-		
rs1303	TT	28	51.9846	18.16920		
	TC	24	56.1262	14.39423	.987	.379
	CC	5	44.5860	28.26926		
rs1800450	CC	43	54.0272	17.61712		
	CT	13	49.4646	18.72979	.387	.681
	TT	-	-	-		
rs5030737	GG	49	52.1210	17.77384		
	GA	8	58.9500	16.93674	1.027	.315
	AA	-	-	-		
rs7041	GG	21	52.8248	14.42236		
	GT	27	50.0619	19.52526	1.785	.178
	TT	9	62.7267	17.08540		

*Calculated using One-way ANOVA

†Significant different from CT

#Significant different from AA and GG

SNP- Single Nucleotide Polymorphism

Table S 14-Associations between Fev1pp and genetic variants

SNP	Genotype	N	Mean	Std. Deviation	F.	p-value*
rs10011792	CC	37	34.7081	9.00723		
	CT	22	32.0773	7.84905	1.291	.261
	TT	-	-	-		
rs1042522	CC	5	34.0000	6.08276		
	CG	25	34.2680	7.84298	.039	.962
	GG	30	33.6067	9.83414		
rs1042713	CC†	21	29.8190	8.97260		
	TC	24	36.4583	7.80735	3.836	.027
	TT	14	34.9071	7.73886		
rs1042714	GG	30	34.6967	7.63108		
	CG	24	33.3333	9.64440	.727	.488
	CC	5	29.8000	9.65401		
rs1042717	CC	31	35.3129	8.03462		
	CT	25	32.2400	9.70945	.869	.425
	TT	4	33.5500	5.90000		
rs10461985	GG	56	34.0750	8.83065		
	GA	4	31.6750	6.44121	.283	.597
	AA	-	-	-		
rs1051303	CC	8	31.8750	10.60239		
	TC	35	34.1114	7.15767	.214	.808
	TT	16	33.8125	10.79641		
rs10516526	GG	50	33.6580	8.78250		
	GA	10	35.2000	8.40370	.260	.612
	AA	-	-	-		
rs1051740	AA	41	33.8780	8.77267		
	AG	16	33.2438	8.94867	.038	.963
	GG	2	34.5000	4.94975		
rs1059823	TT	14	35.2143	11.03267		
	TC	26	32.3808	7.43548	.714	.494
	CC	20	35.0000	8.46665		
rs10844154	TT	10	33.1000	8.81224		
	TG	35	35.7114	8.47803	2.211	.119
	GG	15	30.2667	8.33638		
rs11001819	GG	23	32.0304	9.30999		
	GA	27	35.7852	8.47438	1.214	.304
	AA	10	33.2000	7.29992		
rs11046966	AA	30	32.1067	8.67954		
	AG	23	35.3783	8.68489	1.402	.254
	GG	7	36.8571	7.98809		
RS11172113	AA	9	36.3333	10.73546		
	AG	22	33.3727	7.32440	.407	.668
	GG	29	33.5759	9.11018		
rs1129055	GG	37	33.2946	8.44906		
	GA	19	35.0526	8.72718	.255	.776
	AA	-	-	-		
rs1130866	TT	13	32.0769	7.54389		
	TC	37	34.1324	8.84226	.462	.632
	CC	10	35.5000	9.81212		
rs1131620	CC	9	33.3333	10.83974		
	CT	35	34.1114	7.15767	.029	.971
	TT	16	33.8125	10.79641		
rs1138272	CC	53	33.8660	8.72924		
	CT	5	32.8000	9.14877	.068	.796
	TT	-	-	-		
rs1143634	GG	36	35.4167	8.33024		
	GA	19	30.2053	8.18783	2.799	.069
	AA	5	37.2000	10.03494		
rs1155563	CC	2	34.0000	9.89949		
	CT	25	33.8880	9.39842	.001	.999
	TT	30	33.9900	7.85886		
rs11556218	TT	46	33.2000	8.63944		
	TG	14	36.2643	8.66164	1.349	.250
	GG	-	-	-		

rs11614913	CC	30	33.8067	9.45428		
	CT	24	34.4042	7.83296	.117	.890
	TT	6	32.5000	9.09395		
rs11677877	TT	53	33.6453	8.77669		
	TG	6	34.4500	7.75725	.046	.831
	GG	-	-	-		
rs11739136	GG	47	33.8277	8.61613		
	GA	13	34.2308	9.21189	.022	.884
	AA	-	-	-		
rs12477314	GG	39	33.4333	8.99679		
	GA	19	34.4737	8.36905	.314	.732
	AA	2	38.0000	7.07107		
rs12504628	TT	39	33.4333	8.99679		
	TC	19	34.4737	8.36905	.314	.732
	CC	2	38.0000	7.07107		
rs12899618	CC	46	34.0413	8.64645		
	CT	14	33.5000	9.06175	.041	.840
	TT	-	-	-		
rs12922394	GG	56	33.9982	8.68680		
	GA	4	32.7500	9.63933	.076	.784
	AA	-	-	-		
rs1303	TT	28	34.1750	9.54225		
	TC	27	33.1481	8.56515	.350	.706
	CC	5	36.6000	1.94936		
rs1800450	CC	44	33.3614	8.28052		
	CT	15	34.5333	9.41023	1.679	.196
	TT	-	-	-		
rs5030737	GG#	52	33.8596	8.73023		
	GA	8	34.2750	8.83690	.016	.901
	AA	-	-	-		
rs7041	GG	22	32.6227	8.37615		
	GT	29	34.8000	8.07925	.393	.677
	TT	9	34.2222	11.58423		

*Calculated using One-way ANOVA

†Significant different from CT

SNP- Single Nucleotide Polymorphism

Table S 15-Associations between FVCpp and genetic variants

SNP	Genotype	N	Mean	Std. Deviation	F.	p-value*
rs10011792	CC	37	62.4757	16.49056		
	CT	22	63.8818	15.37291	.105	.747
	TT	-	-	-		
rs1042522	CC	5	1.8820	.80732		
	CG	25	2.3700	.64972	1.325	.274
	GG	30	2.1543	.70556		
rs1042713	CC	21	60.0762	18.70620		
	CT	24	62.7917	15.27140	.971	.385
	TT	14	67.7429	12.15931		
rs1042714	GG	30	65.4000	14.71467		
	CG	24	57.7500	16.44556	2.970	.059
	CC	5	73.8000	14.68673		
rs1042717	CC†	31	67.2710	12.99164		
	CT	25	57.0000	17.65408	3.436	.039
	TT	4	68.6500	14.92503		
rs10461985	GG	56	62.7071	16.17527		
	GA	4	68.3500	9.92421	.469	.496
	AA	-	-	-		
rs1051303	CC	8	63.6250	16.54377		
	TC	35	65.4000	14.52009	1.392	.257
	TT	16	57.4375	18.25000		
rs10516526	GG	50	61.6800	15.11102		
	GA	10	70.1000	18.33303	2.411	.126
	AA	-	-	-		
rs1051740	AA	41	62.0244	15.45071		
	AG	16	66.6875	17.28459	.853	.431
	GG	2	53.5000	17.67767		
rs1059823	TT	14	61.0714	18.68963		
	TC	26	60.0000	14.82150	1.824	.171
	CC	20	68.5000	14.29207		
rs10844154	TT	10	55.8000	13.41475		
	TG	35	63.5429	14.77140	1.526	.226
	GG	15	66.8667	18.92416		
rs11001819	GG	23	58.4957	15.95777		
	GA	27	66.5778	14.93106	1.685	.195
	AA	10	64.2000	16.92992		
rs11046966	AA	30	62.2200	16.83562		
	AG	23	61.1043	14.91538	1.717	.189
	GG	7	73.2857	11.89838		
rs11172113	AA	9	60.5556	18.62868		
	AG	22	64.7545	16.47254	.244	.784
	GG	29	62.6000	14.88566		
rs1129055	GG	37	62.0541	17.68695		
	GA	19	65.5789	12.76852	.349	.707
	AA	-	-	-		
rs1130866	TT	13	70.1538	11.66795		
	TC	37	62.3243	16.40971	2.240	.116
	CC	10	56.7000	16.32347		
rs1131620	CC	9	64.1111	15.54384		
	CT	35	65.4000	14.52009	1.430	.248
	TT	16	57.4375	18.25000		
rs1138272	CC	53	63.4717	15.48394		
	CT	5	57.2000	23.00435	.690	.410
	TT	-	-	-		
rs1143634	GG	36	63.8056	15.94960		
	GA	19	62.3158	15.89204	.108	.898
	AA	5	60.8000	18.01943		
rs1155563	CC	2	45.5000	16.26346		
	CT	25	62.3440	15.09167	1.614	.208
	TT	30	65.4133	16.06692		
rs11556218	TT	46	62.4478	15.28539		
	TG	14	65.1714	17.99775	.314	.578
	GG	-	-	-		

rs11614913	CC	30	63.0533	16.05258		
	CT	24	61.9333	16.07273	.325	.724
	TT	6	67.8333	15.63863		
rs11677877	TT	53	62.8415	16.12787		
	TG	6	64.4000	15.75563	.051	.823
	GG	-	-	-		
rs11739136	GG	47	62.4043	16.14960		
	GA	13	65.5385	15.02007	.395	.532
	AA	-	-	-		
rs12477314	GG	39	60.1282	16.24506		
	GA	19	69.0000	14.45683	2.086	.134
	AA	2	64.5000	2.12132		
rs12504628	TT	39	60.1282	16.24506		
	TC	19	69.0000	14.45683	2.086	.134
	CC	2	64.5000	2.12132		
rs12899618	CC	46	62.6957	14.70273		
	CT	14	64.3571	19.70399	.116	.734
	TT	-	-	-		
rs12922394	GG	56	62.7321	15.78083		
	GA	4	68.0000	18.29390	.409	.525
	AA	-	-	-		
rs1303	TT	28	62.0000	16.81261		
	TC	27	63.8889	14.90311	.126	.882
	CC	5	64.8000	18.25377		
rs1800450	CC	44	62.7955	15.04186		
	CT	15	64.1333	18.98445	.057	.944
	TT	-	-	-		
rs5030737#	GG	52	65.2000	14.88866		
	GA	8	49.3250	15.82004	7.762	.007
	AA	-	-	-		
rs7041	GG	22	66.7909	16.14488		
	GT	29	62.5724	15.82003	1.639	.203
	TT	9	55.6667	13.77498		

*Calculated using One-way ANOVA

†Significant different from CT

#Significant different from GA

SNP- Single Nucleotide Polymorphism

Table S 16-Associations between FEV1/FVC ratio and genetic variants

SNP	Genotype	N	Mean	Std. Deviation	F.	p-value*
rs10011792	CC	37	48.0000	20.88460		
	CT	22	40.9595	8.71506	2.254	.139
	TT	-	-	-		
rs1042522	CC	5	57.0000	32.57299		
	CG	25	42.8444	12.73281	1.393	.257
	GG	30	45.7000	17.64428		
rs1042713	CC	21	42.5238	16.66919		
	CT	24	50.0000	20.82223	1.425	.249
	TT	14	41.7221	11.02700		
rs1042714	GG	30	45.6037	18.19077		
	CG	24	48.0417	17.46171	1.961	.150
	CC	5	31.2000	6.97854		
rs1042717	CC	31	42.6810	14.26702		
	CT	25	49.4800	20.95654	1.150	.324
	TT	4	41.7500	14.79583		
rs10461985	GG	56	46.0714	17.90465		
	GA	4	36.7775	3.98807	1.058	.308
	AA	-	-	-		
rs1051303	CC	8	43.0000	11.42679		
	TC	35	43.5460	16.41749	.955	.391
	TT	16	50.5625	22.07553		
rs10516526	GG	50	46.4222	18.46509		
	GA	10	40.6000	10.60608	.925	.340
	AA	-	-	-		
rs1051740	AA	41	61.6098	23.98320		
	AG	13	70.7692	25.66500	1.034	.362
	GG	2	97.0000	2.82843		
rs1059823	TT	14	51.7857	23.52798		
	TC	26	45.8119	17.85387	1.757	.182
	CC	20	40.5500	9.70879		
rs10844154	TT	10	48.5000	16.44013		
	TG	35	47.4603	18.10370	1.519	.228
	GG	15	38.7333	15.84508		
rs11001819	GG	23	45.1352	17.86277		
	GA	27	45.1481	16.96309	.046	.955
	AA	10	47.0000	19.64123		
rs11046966	AA	30	44.2333	15.25797		
	AG	23	48.3961	21.61847	.619	.542
	GG	7	41.0000	9.88264		
rs11172113	AA	9	52.4444	23.33512		
	AG	22	43.7727	14.93775	.857	.430
	GG	29	44.5555	17.35596		
rs1129055	GG	37	46.9759	20.26470		
	GA	19	42.8421	11.80519	.364	.697
	AA	-	-	-		
rs1130866	TT	13	36.7692	9.69668		
	TC	37	46.1381	19.21604	3.094	.053
	CC	10	54.2000	14.21111		
rs1131620	CC	9	43.7778	10.94050		
	CT	35	43.5460	16.41749	.932	.400
	TT	16	50.5625	22.07553		
rs1138272	CC	53	44.8700	17.32454		
	CT	5	53.0000	22.02272	.964	.330
	TT	-	-	-		
rs1143634	GG	36	46.8056	16.68501		
	GA	19	39.2689	11.88860	3.044	.055
	AA	5	59.2000	31.53094		
rs1155563	CC	2	75.0000	52.32590		
	CT	25	45.3200	13.13938	3.075	.054
	TT	30	43.8037	17.77466		
rs11556218	TT	46	44.0870	16.17244		
	TG	14	49.9364	21.24037	1.208	.276
	GG	-	-	-		

rs11614913	CC	30	44.9667	15.82334		
	CT	24	47.0879	20.31762	.277	.759
	TT	6	41.3333	14.65151		
rs11677877	TT	53	45.3208	17.75774		
	TG	6	45.8517	17.78651	.005	.945
	GG	-	-	-		
rs11739136	GG	47	45.5981	18.73751		
	GA	13	44.9231	12.40606	.015	.903
	AA	-	-	-		
rs12477314	GG	39	47.5926	18.97368		
	GA	19	41.0526	14.31966	.892	.415
	AA	2	45.5000	9.19239		
rs12504628	TT	39	47.5926	18.97368		
	TC	19	41.0526	14.31966	.892	.415
	CC	2	45.5000	9.19239		
rs12899618	CC	46	45.5459	16.58677		
	CT	14	45.1429	20.79148	.006	.941
	TT	-	-	-		
rs12922394	GG	56	45.5020	16.70114		
	GA	4	44.7500	29.76995	.007	.935
	AA	-	-	-		
rs1303	TT	28	47.7896	19.92509		
	TC	27	42.6667	15.64510	.617	.543
	CC	5	47.4000	11.58879		
rs1800450	CC	44	45.3661	19.27358		
	CT	15	44.4000	10.78888	.646	.528
	TT	-	-	-		
rs5030737	GG†	52	42.2713	13.32253		
	GA	8	66.1250	26.73915	16.278	.000
	AA	-	-	-		
rs7041	GG	22	41.1414	19.72018		
	GT	29	46.2759	12.48970	1.654	.200
	TT	9	53.3333	23.66432		

*Calculated using One-way ANOVA

†Significant different from GA

SNP- Single Nucleotide Polymorphism

Table S 17-Associations between maximum inspiratory pressure and genetic variants

SNP	Genotype	N	Mean	Std. Deviation	F.	p-value*
rs10011792	CC	35	66.9143	26.64844		
	CT	21	61.8095	21.66245	.551	.461
	TT	-	-	-		
rs1042522	CC	5	69.2000	20.96902		
	CG	24	65.3750	19.95171	.079	.924
	GG	28	64.3929	29.20216		
rs1042713	CC	19	54.1579	26.25995		
	CT	24	70.1667	25.60854	2.948	.061
	TT	13	71.3077	15.77120		
rs1042714	GG	28	66.0000	22.69116		
	CG	24	65.9583	28.22654	.559	.575
	CC	4	52.2500	17.74589		
rs1042717	CC	29	70.2069	19.60390		
	CT	25	61.8000	29.63950	1.830	.170
	TT	3	45.6667	4.04145		
rs10461985	GG	53	65.4906	25.40792		
	GA	4	61.7500	11.78629	.084	.773
	AA	-	-	-		
rs1051303	CC	8	63.8750	11.06394		
	TC	33	69.3030	22.95578	1.488	.235
	TT	15	56.1333	32.01755		
rs10516526	GG	47	64.4681	24.34878		
	GA	10	68.8000	27.10392	.251	.618
	AA	-	-	-		
rs1051740	AA	41	61.6098	23.98320		
	AG	13	70.7692	25.66500	2.530	.089
	GG	2	97.0000	2.82843		
rs1059823	TT	13	64.0769	20.91834		
	TC	24	57.9583	24.03707	2.686	.077
	CC	20	74.7000	25.56540		
rs10844154	TT	10	52.0000	20.77926		
	TG	32	66.4063	21.47145	2.040	.140
	GG	15	71.5333	31.01812		
rs11001819	GG	23	64.8261	24.56467		
	GA	25	69.9200	23.49986	1.553	.221
	AA	9	53.2222	26.59313		
rs11046966	AA	27	67.0370	28.91164		
	AG	23	64.6957	21.92017	.229	.796
	GG	7	60.0000	15.53491		
rs11172113	AA	9	78.3333	30.72458		
	AG	20	66.2500	23.08309	1.912	.158
	GG	28	60.2857	22.86167		
rs1129055	GG	34	63.7647	23.14913		
	GA	19	68.1053	28.76320	.189	.829
	AA	-	-	-		
rs1130866	TT	12	60.0000	17.09864		
	TC	36	63.2500	27.41259	2.106	.132
	CC	9	80.1111	16.04248		
rs1131620	CC	9	65.4444	11.37004		
	CT	33	69.3030	22.95578	1.498	.233
	TT	15	56.1333	32.01755		
rs1138272	CC	50	65.6200	25.25372		
	CT	5	63.6000	22.45662	.030	.864
	TT	-	-	-		
rs1143634	GG	35	67.6571	24.18306		
	GA	17	56.0000	22.78431	2.314	.109
	AA	5	79.6000	28.11227		
rs1155563	CC	2	53.0000	8.48528		
	CT	23	68.6522	20.16660	.476	.624
	TT	29	64.3793	27.82780		
rs11556218	TT	44	64.1136	23.83199		
	TG	13	69.0000	27.95234	.390	.535
	GG	-	-	-		

rs11614913	CC	29	68.5862	27.63993		
	CT	24	62.4167	18.36565	.600	.552
	TT	4	57.7500	37.18759		
rs11677877	TT	50	65.3400	25.02212		
	TG	6	62.1667	25.05527	.086	.770
	GG	-	-	-		
rs11739136	GG	44	64.9318	26.06021		
	GA	13	66.2308	20.03810	.027	.869
	AA	-	-	-		
rs12477314	GG	37	66.5946	27.49289		
	GA	18	63.6667	19.44827	.293	.747
	AA	2	54.0000	5.65685		
rs12504628	TT	37	66.5946	27.49289		
	TC	18	63.6667	19.44827	.293	.747
	CC	2	54.0000	5.65685		
rs12899618	CC	43	65.7209	26.37759		
	CT	14	63.7143	19.16097	.069	.794
	TT	-	-	-		
rs12922394	GG	53	66.3962	24.75056		
	GA	4	49.7500	19.60230	1.717	.195
	AA	-	-	-		
rs1303	TT	26	66.8846	26.23483		
	TC	26	61.5769	23.34639	.780	.463
	CC	5	75.6000	23.71287		
rs1800450	CC	42	66.8571	23.92832		
	CT	14	58.2143	26.13038	1.408	.254
	TT	-	-	-		
rs5030737	GG	50	66.0000	24.79302		
	GA	7	59.7143	24.75018	.395	.532
	AA	-	-	-		
rs7041	GG	21	62.4286	22.60657		
	GT	27	68.0370	27.13851	.329	.721
	TT	9	63.3333	23.07054		

*Calculated using One-way ANOVA

SNP- Single Nucleotide Polymorphism

Table S 18-Associations between maximum expiratory pressure and genetic variants

SNP	Genotype	N	Mean	Std. Deviation	F.	p-value*
rs10011792	CC	35	114.2000	39.00890		
	CT	21	105.7619	42.70820	.572	.453
	TT	-	-	-		
rs1042522	CC	5	116.4000	45.50604		
	CG	24	111.5833	40.84000	.104	.902
	GG	28	108.2143	40.21089		
rs1042713	CC	19	98.7895	49.43636		
	CT	24	117.8333	32.95671	1.353	.267
	TT	13	116.3846	36.34221		
rs1042714	GG	28	112.1429	36.18954		
	CG	24	108.9583	45.64083	.068	.935
	CC	4	115.7500	43.40795		
rs1042717	CC	29	117.0690	34.20937		
	CT	25	107.0800	46.33026	1.858	0.166
	TT	3	72.6667	16.25833		
rs10461985	GG	53	109.5472	40.80609		
	GA	4	121.0000	34.51570	.298	.588
	AA	-	-	-		
rS1051303	CC	8	114.5000	28.79980		
	TC	33	117.0000	39.98515	1.452	.243
	TT	15	96.0667	44.36290		
rs10516526	GG	47	110.8936	42.26893		
	GA	10	107.8000	30.52067	.048	.828
	AA	-	-	-		
rs1051740	AA	41	104.8780	38.36613		
	AG	13	127.6923	44.79097	1.846	.168
	GG	2	129.0000	19.79899		
rs1059823	TT	13	111.3846	23.93581		
	TC	24	101.8750	43.90039	1.098	.341
	CC	20	119.8500	43.47083		
rs10844154	TT	10	111.7000	41.62812		
	TG	32	108.3438	38.12425	.095	.909
	GG	15	113.7333	46.04108		
rs11001819	GG	23	113.6522	44.96424		
	GA	25	112.3200	35.85819	.637	.533
	AA	9	96.4444	40.65437		
rs11046966	AA	27	111.1852	39.86326		
	AG	23	112.8261	44.99662	.320	.728
	GG	7	99.0000	24.92656		
rs11172113	AA	9	109.2222	22.87891		
	AG	20	123.2000	45.71030	1.740	.185
	GG	28	101.5357	39.09768		
rs1129055	GG	34	105.9118	41.61539		
	GA	19	116.0526	40.38904	.529	.592
	AA	-	-	-		
rs1130866	TT	12	103.9167	33.23596		
	TC†	36	104.6667	41.19015	3.532	.036
	CC	9	141.6667	32.84433		
rs1131620	CC	9	109.7778	30.43755		
	CT	33	117.0000	39.98515	1.418	.251
	TT	15	96.0667	44.36290		
rs1138272	CC	50	109.7400	41.25372		
	CT	5	118.6000	34.14381	.215	.645
	TT	-	-	-		
rs1143634	GG	35	112.9714	42.51954		
	GA	17	106.9412	40.25151	.200	.819
	AA	5	103.6000	25.45192		
rs1155563	CC	2	93.0000	48.08326		
	CT	23	119.1739	30.58171	.800	.455
	TT	29	107.5862	44.65304		
rs11556218	TT	44	109.0909	38.37624		
	TG	13	114.6154	47.44741	.186	.668
	GG	-	-	-		

rs11614913	CC	29	112.5172	46.41553		
	CT	24	109.7500	31.61212	.219	.804
	TT	4	98.2500	46.94234		
rs11677877	TT	50	113.7800	40.28687		
	TG	6	88.1667	34.95378	2.216	.142
	GG	-	-	-		
rs11739136	GG	44	109.0227	42.23879		
	GA	13	114.8462	33.65473	.207	.651
	AA	-	-	-		
rs12477314	GG	37	107.4865	42.02025		
	GA	18	113.1667	38.42984	.601	.552
	AA	2	138.0000	1.41421		
rs12504628	TT	37	107.4865	42.02025		
	TC	18	113.1667	38.42984	.601	.552
	CC	2	138.0000	1.41421		
rs12899618	CC	43	111.2093	40.98464		
	CT	14	107.7143	39.19464	.078	.781
	TT	-	-	-		
rs12922394	GG	53	111.1509	40.67762		
	GA	4	99.7500	37.07088	.295	.589
	AA	-	-	-		
rs1303	TT	26	110.6154	41.11163		
	TC	26	102.9615	33.64578	2.716	.075
	CC	5	147.4000	54.33507		
rs1800450	CC	42	111.7381	38.64350		
	CT	14	106.2857	47.31958	.094	.911
	TT	-	-	-		
rs5030737	GG	50	110.3200	40.76635		
	GA	7	110.5714	39.19123	.000	.988
	AA	-	-	-		
rs7041	GG	21	114.6667	41.14527		
	GT	27	110.4815	39.58474	.416	.662
	TT	9	99.8889	42.82652		

*Calculated using One-way ANOVA

†Significant different from CC

SNP- Single Nucleotide Polymorphism

Table S 19-Associations between quadriceps muscle strength (dynamometer) and genetic variants

SNP	Genotype	N	Mean	Std. Deviation	F.	p-value*
rs10011792	CC	35	22.9600	9.57202		
	CT	21	20.4091	9.22109	.987	.325
	TT	-	-	-		
rs1042522	CC	5	21.5200	8.53710		
	CG	24	22.5333	11.02817	.100	.905
	GG	28	21.3793	8.27912		
rs1042713	. CC†	19	17.8737	9.44051		
	CT	24	25.0667	9.76287	3.346	.043
	TT	14	22.2429	9.43843		
rs1042714	GG	28	21.4233	7.09807		
	CG	24	22.1043	11.19474	.305	.738
	CC	4	25.3750	15.43554		
rs1042717	CC#	29	25.1967	9.96883		
	CT	25	18.3250	7.57400	4.363	.017
	TT	3	18.1750	6.74802		
rs10461985	GG	53	21.4556	9.48883		
	GA	4	27.4500	6.33377	1.532	.221
	AA	-	-	-		
rS1051303	CC	8	23.9125	14.02197		
	TC	33	23.5061	8.74325	2.224	.118
	TT	15	17.8500	7.24127		
rs10516526	GG	47	21.0673	9.45748		
	GA	10	26.2333	8.13910	2.356	.130
	AA	-	-	-		
rs1051740	AA	41	21.5625	10.35695		
	AG	13	22.8533	7.40809	.130	.878
	GG	2	23.6500	3.69624		
rs1059823	TT	13	22.5308	10.92439		
	TC	24	20.4840	8.98279	.487	.617
	CC	20	23.1700	9.09020		
rs10844154	TT	10	20.8300	8.16851		
	TG	32	21.5818	9.17410	.220	.804
	GG	15	23.1933	10.99542		
rs11001819	GG	23	19.9000	7.72540		
	GA	25	24.2115	10.66330	1.494	.233
	AA	9	20.1333	8.74343		
rs11046966	AA	27	21.9103	8.99942		
	AG	23	21.2045	9.55089	.195	.823
	GG	7	23.7857	11.60021		
rs11172113	AA	9	19.8556	8.80158		
	AG	20	25.2429	10.92221	2.216	.119
	GG	28	19.9857	7.80720		
rs1129055	GG	34	20.6557	8.46044		
	GA	19	25.4684	10.63313	3.333	.074
	AA	-	-	-		
rs1130866	TT	12	25.4000	11.69622		
	TC	36	19.6111	7.95188	2.935	.062
	CC	9	25.8000	9.26512		
rs1131620	CC	9	23.0111	13.39220		
	CT	33	23.5061	8.74325	2.113	.131
	TT	15	17.8500	7.24127		
rs1138272	CC	50	22.7216	9.62475		
	CT	5	16.6600	3.72733	1.977	.171
	TT	-	-	-		
rs1143634	GG	35	22.0889	9.73425		
	GA	17	22.0765	9.42122	.158	.854
	AA	5	19.5800	8.07527		
rs1155563	CC	2	19.1500	12.51579		
	CT	23	21.8220	9.03919	.194	.825
	TT	29	22.8857	9.86069		
rs11556218	TT	44	22.0022	9.79444		
	TG	13	21.4077	8.17338	.040	.843
	GG	-	-	-		

rs11614913	CC	29	24.4034	11.05174		
	CT	24	19.0375	6.42582	2.280	.112
	TT	4	20.7600	8.24154		
rs11677877	TT	50	21.6902	9.27244		
	TG	6	24.4000	11.40509	.438	.511
	GG	-	-	-		
rs11739136	GG	44	21.4674	9.06449		
	GA	13	23.4083	10.84146	.402	.528
	AA	-	-	-		
rs12477314	GG	37	21.8053	9.05992		
	GA	18	21.8722	10.55818	.016	.984
	AA	2	23.0500	9.54594		
rs12504628	TT	37	21.5433	8.03667		
	TC	18	21.9333	10.20199	.111	.895
	CC	2	23.9250	15.69594		
rs12899618	CC	43	21.8886	8.45000		
	CT	14	21.8071	12.26568	.001	.978
	TT	-	-	-		
rs12922394	GG	53	21.6389	9.37855		
	GA	4	24.9750	10.37638	.466	.498
	AA	-	-	-		
rs1303	TT	26	22.3714	9.55831		
	TC	26	20.9920	10.09467	.213	.809
	CC	5	23.4400	4.33394		
rs1800450	CC	42	22.1932	9.10462		
	CT	14	20.6923	10.94703	.249	.620
	TT	-	-	-		
rs5030737	GG	50	22.5260	9.73550		
	GA	7	17.7625	5.67550	1.800	.185
	AA	-	-	-		
rs7041	GG	21	21.1429	8.15657		
	GT	27	23.8036	10.52723	1.648	.202
	TT	9	17.5444	7.23880		

*Calculated using One-way ANOVA

†Significant different from CT

#Significant different from CT

SNP- Single Nucleotide Polymorphism

Table S 20-Associations between handgrip and genetic variants

SNP	Genotype	N	Mean	Std. Deviation	F.	p-value*
rs10011792	CC	26	38.0962	9.05982		
	CT	14	38.2857	7.96559	.004	.948
	TT	-	-	-		
rs1042522	CC	2	37.5000	2.12132		
	CG	17	37.5588	8.93358	.015	.985
	GG	22	38.0455	9.22975		
rs1042713	CC	14	34.9643	9.50398		
	TC	19	40.5789	8.08507	1.794	.180
	TT	7	38.0000	6.73300		
rs1042714	GG	18	40.2222	7.62006		
	CG	19	36.3421	9.41638	.956	.394
	CC	3	37.3333	8.73689		
rs1042717	CC	19	38.4211	7.15942		
	CT	21	37.2143	10.33026	.099	.906
	TT	1	39.0000	.		
rs10461985	GG	37	37.4730	8.51139		
	GA	4	41.0000	11.83216	.578	.452
	AA	-	-	-		
rs1051303	CC	6	37.7500	8.04829		
	TC	24	40.1667	7.38781	2.198	.125
	TT	10	33.6000	10.50079		
rs10516526	GG	35	37.1286	9.11797		
	GA	6	41.8333	5.15429	1.494	.229
	AA	-	-	-		
rs1051740	AA	26	36.4423	7.26681		
	AG	13	42.0769	10.17727	2.267	.118
	GG	1	32.0000	-		
rs1059823	TT	10	39.3500	9.70123		
	TC	18	35.4444	8.22637	1.200	.312
	CC	13	39.9231	8.62614		
rs10844154	TT	8	37.8750	10.03476		
	TG	21	36.5952	9.08518	.536	.590
	GG	12	39.9167	7.58537		
rs11001819	GG	19	35.9737	9.95891		
	GA	14	41.3571	6.22164	1.804	.178
	AA	8	36.0000	8.60233		
rs11046966	AA	20	39.8000	8.25642		
	AG	16	34.0313	9.31033	2.806	.073
	GG	5	42.0000	4.52769		
rs11172113	AA	8	36.6875	11.02898		
	. AG†	17	42.3529	7.22791	5.093	.011
	GG	16	33.5625	7.00446		
rs1129055	GG	23	37.6087	7.55428		
	GA	16	39.3125	10.10425	1.464	.244
	AA	-	-	-		
rs1130866	TT	9	38.4444	4.90181		
	TC	25	36.8200	10.24581	.518	.600
	CC	7	40.5714	6.82781		
rs1131620	CC	7	35.7857	8.99934		
	CT	24	40.1667	7.38781	2.358	.108
	TT	10	33.6000	10.50079		
rs1138272	CC	35	38.2143	9.00922		
	CT	4	38.7500	5.73730	.013	.909
	TT	-	-	-		
rs1143634	GG	25	39.2800	8.60484		
	GA	13	33.9615	8.46183	2.116	.134
	AA	3	42.3333	8.02081		
rs1155563	CC	-	-	-		
	CT	15	38.8333	7.66175	.055	.816
	TT	23	38.1739	8.92724		
rs11556218	. TT#	31	36.0484	8.13875		
	TG	10	43.3000	8.74389	5.796	.021
	GG	-	-	-		

rs11614913	CC	20	39.8500	9.90096		
	CT	16	34.9688	5.92303	1.446	.248
	TT	5	38.8000	10.80278		
rs11677877	TT	36	38.9028	8.74546		
	TG	4	31.5000	1.00000	2.797	.103
	GG	-	-	-		
rs11739136	GG	32	37.7344	9.79630		
	GA	9	38.1111	3.48010	.013	.911
	AA	-	-	-		
rs12477314	GG	27	37.7222	9.47906		
	GA	12	38.1667	8.06602	.019	.981
	AA	2	37.0000	4.24264		
rs12504628	TT	27	37.7222	9.47906		
	TC	12	38.1667	8.06602	.019	.981
	CC	2	37.0000	4.24264		
rs12899618	CC	36	37.9167	8.68620		
	CT	5	37.1000	10.35857	.037	.848
	TT	-	-	-		
rs12922394	GG	37	38.2568	9.10862		
	GA	4	33.7500	2.06155	.953	.335
	AA	-	-	-		
rs1303	TT	19	37.7105	8.98350		
	TC	18	37.5556	9.01125	.079	.924
	CC	4	39.5000	8.69866		
rs1800450	CC	28	38.2679	9.72062		
	CT	13	36.8462	6.47876	.229	.635
	TT	-	-	-		
rs5030737	GG	36	38.1528	9.24648		
	GA	5	35.4000	3.43511	.427	.517
	AA	-	-	-		
rs7041	GG	19	37.7632	9.14143		
	GT	17	38.8235	8.06408	.437	.649
	TT	5	34.6000	10.71448		

*Calculated using One-way ANOVA

†Significant different from GG

#Significant different from TG

SNP- Single Nucleotide Polymorphism

Table S 21-Associations between five time sit-to-stand test and genetic variants

SNP	Genotype	N	Mean	Std. Deviation	F.	p-value*
rs10011792	CC	32	9.8131	5.48420		
	CT	20	9.6820	3.85757	.009	.926
	TT	-	-	-		
rs1042522	CC	4	6.6650	1.67353		
	CG	22	10.8714	6.61335	.104	.216
	GG	27	9.2448	2.90701		
rs1042713	CC	16	11.7375	7.46470		
	TC	22	9.1668	3.22202	2.069	.137
	TT	14	8.4421	2.26784		
rs1042714	GG	28	8.3968	2.59463		
	CG	19	11.5047	7.11243	2.565	.087
	CC	5	10.7920	1.39591		
rs1042717	CC	30	9.3327	2.67306		
	CT	20	10.6190	7.13228	.695	.504
	TT	3	7.6933	2.49588		
rs10461985	GG	49	9.8782	4.98221		
	GA	4	7.8525	2.04340	.643	.426
	AA	-	-	-		
rs1051303	CC	7	13.2714	10.05213		
	TC	31	9.2758	3.56286	2.195	.122
	TT	14	9.0864	3.14151		
rs10516526	GG	44	9.9750	5.08903		
	GA	9	8.5044	3.34279	.685	.412
	AA	-	-	-		
rs1051740	AA	34	10.0544	5.47956		
	AG	16	9.3244	3.71267	.207	.814
	GG	2	8.3100	2.74357		
rs1059823	TT	12	10.5317	8.20241		
	TC	23	10.0639	3.54031	.574	.567
	CC	18	8.7550	3.21733		
rs10844154	TT	9	8.2611	1.97708		
	TG	31	10.2161	5.90080	.568	.570
	GG	13	9.5685	3.11235		
rs11001819	GG	21	10.0343	6.94425		
	GA	24	9.5733	3.13364	.073	.929
	AA	8	9.3700	1.90118		
rs11046966	AA	27	9.5956	3.28722		
	AG	19	10.7553	6.78026	1.237	.299
	GG	7	7.4300	2.97886		
rs11172113	AA	8	12.0875	10.13915		
	AG	20	9.7065	2.91673	1.257	.293
	GG	25	8.9844	3.41291		
rs1129055	GG	32	9.1131	3.47332		
	GA	18	9.4050	3.12920	0.087	.769
	AA	-	-	-		
rs1130866	TT	12	9.4525	3.15823		
	TC	32	10.3281	5.66517	.871	.425
	CC	9	7.9456	3.03127		
rs1131620	CC	8	12.5850	9.50683		
	CT	31	9.2758	3.56286	1.695	.194
	TT	14	9.0864	3.14151		
rs1138272	CC	46	9.4924	4.85884		
	CT	5	10.5140	4.22687	.203	.654
	TT	-	-	-		
rs1143634	GG	33	9.3761	3.62315		
	GA	16	10.5656	6.85557	.338	.715
	AA	4	9.2450	5.09503		
rs1155563	CC	1	10.6200	-		
	CT	23	10.4204	6.44986	.392	.678
	TT	27	9.1896	3.28335		
rs11556218	TT	41	10.0344	5.18835		
	TG	12	8.6692	3.37255	.734	.396
	GG	-	-	-		

rs11614913	CC	27	9.0559	3.38598		
	CT	21	10.2362	6.51100	.595	.555
	TT	5	11.1940	3.34900		
rs11677877	TT	46	9.8107	5.11978		
	TG	6	9.3950	2.64021	.038	.847
	GG	-	-	-		
rs11739136	GG	43	10.0170	5.05816		
	GA	10	8.4710	3.72999	.824	.368
	AA	-	-	-		
rs12477314	GG	33	9.4521	5.87865		
	GA	19	10.2032	2.43241	.140	.869
	AA	1	9.6600	-		
rs12504628	TT	33	9.4521	5.87865		
	TC	19	10.2032	2.43241	.140	.869
	CC	1	9.6600	-		
rs12899618	. CC†	40	8.8408	3.26219		
	CT	13	12.4469	7.50861	5.961	.018
	TT	-	-	-		
rs12922394	GG	49	9.7582	5.03594		
	GA	4	9.3225	.62222	.029	.865
	AA	-	-	-		
rs1303	TT	26	9.7423	6.20869		
	TC	22	9.9036	3.29286	.093	.911
	CC	5	8.8520	2.41140		
rs1800450	CC	41	9.6856	4.88364		
	CT	11	10.1518	5.03704	.237	.790
	TT	-	-	-		
rs5030737	GG	48	9.9735	5.01492		
	GA	5	7.3420	1.20348	1.346	.251
	AA	-	-	-		
rs7041	GG	21	10.2810	6.45991		
	GT	25	9.3008	3.30185	.231	.795
	TT	7	9.5743	4.31599		

*Calculated using One-way ANOVA

†Significant different from CT

SNP- Single Nucleotide Polymorphism

Table S 22-Associations between one minute sit-to-stand test and genetic variants

SNP	Genotype	N	Median	IQR	Chi-Square	p-value*
rs10011792	CC	32	28.5000	22 to 32.75		
	CT	20	26.0000	18.50 to 33.75	0.109	0.742
	TT	-	-	-		
rs1042522	CC	4	30.0000	22 to 34.25		
	CG	22	24.0000	16.50 to 31.25	3.233	0.199
	GG	27	29.0000	22 to 35		
rs1042713	CC	21	22.0000	14.25 to 31		
	TC	24	28.5000	21.75 to 33	4.763	0.092
	TT	14	30.5000	24 to 35		
rs1042714	GG	27	31.0000	24 to 35		
	CG	20	24.0000	17.25 to 31	6.672	.038
	CC	5	23.0000	15 to 25.50		
rs1042717	CC	30	26.0000	21.75 to 33.25		
	CT	21	26.0000	16 to 31.50	2.989	0.224
	TT	2	-	-		
rs10461985	GG	49	26.0000	20.50 to 32.50		
	GA	4	32.5000	23 to 36.75	1-057	.304
	AA	-	-	-		
rs1051303	CC	6	22.5000	17 to 34.25		
	TC	32	29.0000	9.22 to 34.50	2.558	.278
	TT	14	26.5000	16.75 to 31.75		
rs10516526	GG	43	26.0000	20 to 33		
	GA	10	26.5000	20.25 to 37	.081	.776
	AA	-	-	-		
rs1051740	AA	34	25.5000	20 to 32.50		
	AG	16	29.5000	22 to 34.50	.854	.652
	GG	2	28.5000	26 to -		
rs1059823	TT	12	28.5000	23.75 to 35		
	TC	23	21.0000	16 to 33	3.329	.189
	CC	18	29.0000	24 to 33.75		
rs10844154	TT	9	29.0000	21 to 35		
	TG	31	28.0000	21 to 33	.423	.809
	GG	13	25.0000	18 to 33.50		
rs11001819	GG	21	29.0000	22 to 32		
	GA	24	27.5000	20.25 to 35.75	.962	.618
	AA	8	23.0000	20.50 to 30.50		
rs11046966	AA†	27	24.0000	18 to 29		
	AG	20	30.0000	24 to 33.75	8.706	.013
	GG	6	35.5000	29 to 47		
rs11172113	AA	8	32.5000	15.75 to 36.75		
	AG	19	26.0000	22 to 32	1.331	.514
	GG	26	26.0000	17.75 to 32.25		
rs1129055	GG	32	26.0000	20 to 35.75		
	GA	18	27.5000	24 to 31.25	.003	.960
	AA	-	-	-		
rs1130866	TT	12	24.5000	18.50 to 32.75		
	TC	32	26.0000	20.25 to 34.75	.414	.813
	CC	9	29.0000	25.50 to 30		
rs1131620	CC	7	23.0000	20 to 24		
	CT	32	29.0000	22 to 34.50	2.920	.232
	TT	14	26.5000	16.75 to 31.75		*
rs1138272	CC#	46	29.0000	22.75 to 34.25		
	CT	5	20.0000	14 to 21.50	5.258	.012
	TT	-	-	-		
rs1143634	GG	32	29.0000	21.25 to 33.75		
	GA	17	26.0000	18 to 30.50	1.525	.467
	AA	4	32.5000	15.50 to 37.50		
rs1155563	CC	1	24.0000	-		
	CT	22	26.0000	19.50 to 32.75	.587	.746
	TT	28	29.5000	20.50 to 33.75		
rs11556218	TT	41	26.0000	20 to 33.50		
	TG	12	29.0000	22.75 to 32.75	.368	.544
	GG	-	-	-		

rs11614913	CC	28	29.5000	23.25 to 35		
	CT	20	24.5000	21 to 32	4.842	.089
	TT	5	20.0000	12.50 to 26		
rs11677877	TT	46	26.0000	20.75 to 34.25		
	TG	6	30.0000	16.25 to 33	.080	.778
	GG	-	-	-		
rs11739136	GG	42	26.0000	20 to 33.25		
	GA	11	29.0000	24 to 33	.607	.436
	AA	-	-	-		
rs12477314	GG	33	30.0000	24 to 35.50		
	GA	19	23.0000	20 to 29	3.911	.141
	AA	1	20.0000	-		
rs12504628	TT	33	31.0000	24 to 36		
	TC	19	23.5000	17.75 to 29.50	6.397	.041
	CC	1	20.0000	-		
rs12899618	CC	41	28.0000	20.50 to 34.50		
	CT	12	25.0000	18 to 30.50	1.021	.312
	TT	-	-	-		
rs12922394	GG	49	28.0000	20.50 to 33.50		
	GA	4	23.0000	17.50 to 30.75	.601	.438
	AA	-	-	-		
rs1303	TT	26	29.0000	19.50 to 33.50		
	TC	22	26.0000	20.75 to 32.50	.817	.665
	CC	5	32.0000	22.50 to 38		
rs1800450	CC	40	27.0000	22 to 33		
	CT	12	23.5000	14.25 to 38.50	.530	.466
	TT	-	-	.		
rs5030737	GG	48	26.0000	20.25 to 33		
	GA	5	29.0000	21 to 33	.006	.939
	AA	-	-	-		
rs7041	GG	21	29.0000	20.50 to 34.50		
	GT	25	26.0000	20.50 to 32	1.107	.575
	TT	7	24.0000	17 to 29		

*Calculated using Kruskal-Wallis test

†Significant different from AG

#Significant different from CT

SNP- Single Nucleotide Polymorphism

IQR- Interquartile range

Appendix VI: Participant information sheet

Folha de informação ao participante

O Sr./Sra. está a ser convidado/a para participar no estudo de investigação clínica intitulado: "GENIAL – Marcadores genéticos e clínicos na trajetória da DPOC". Mas, antes de decidir, é importante que compreenda porque é que a investigação está a ser realizada e o que é que a mesma envolve. Por favor, leia a informação com atenção e discuta a sua participação com outros, se assim o entender. Se houver algo que não esteja claro para si ou necessitar de informação adicional, por favor pergunte aos investigadores (contactos no final deste documento). Use o tempo que precisar para decidir se deseja ou não participar. Muito obrigado desde já por ler a informação.

Qual é o propósito do estudo?
Este estudo visa determinar o papel das mutações genéticas associadas ao desenvolvimento e trajetória da Doença Pulmonar Obstrutiva Crónica (DPOC) e identificar os marcadores clínicos (e.g., dispneia; número de exacerbações; função pulmonar; tolerância ao exercício) capazes de detetar episódios de exacerbações agudas da DPOC (episódios de agravamento dos sintomas respiratórios que é acima da sua variação normal do dia-a-dia e leva à alteração da medicação).

A suscetibilidade para desenvolver DPOC varia consideravelmente entre indivíduos, sugerindo que outros fatores de risco para além do tabaco (principal fator de risco) podem influenciar o desenvolvimento da doença. Estudos recentes demonstraram que a suscetibilidade genética pode desempenhar um papel determinante na patogénese da DPOC. Contudo, pouco se sabe acerca desta relação entre o desenvolvimento da doença e o perfil genético dos pacientes. Da mesma forma, sabe-se que a deterioração clínica dos pacientes é altamente dependente da frequência e gravidade das exacerbações agudas, e que pacientes com função pulmonar semelhante apresentam níveis diferentes de incapacidade/suscetibilidade às exacerbações agudas. Assim, os resultados deste estudo irão potencialmente contribuir para melhorar o conhecimento sobre a DPOC e informar sobre as estratégias para prevenir, detetar precocemente e gerir as exacerbações agudas. Para que seja possível alcançar estes objetivos vimos então solicitar a sua participação neste estudo que será realizado na Escola Superior de Saúde da Universidade de Aveiro/iBiMED, centros de saúde e centro hospitalar do Baixo Vouga, Centro Hospitalar do Médio Ave, Hospital Pedro Hispano e Hospital Distrital da Figueira da Foz.

Porque é que fui escolhido?
Foi escolhido/a porque é uma pessoa saudável, com doença pulmonar obstrutiva crónica em fase estável ou cuidador/a de uma pessoa com esta doença. Para o estudo, precisamos de dados de aproximadamente 400 pessoas, com uma condição clínica semelhante à sua, que aceitem participar.

Tenho de participar?
A decisão de participar, ou não, é completamente sua. Se decidir participar vai-lhe ser pedido que assine um formulário de consentimento informado mas, é totalmente livre de desistir a qualquer momento, sem que para tal tenha de dar qualquer justificação. A decisão de desistir ou de não participar, não afetará a qualidade dos serviços de saúde ou qualquer outro, que lhe são prestados agora ou no futuro.

O que me acontecerá caso decida participar?
Se decidir participar, após assinar e entregar aos investigadores o consentimento informado, será feita uma avaliação do seu estado de saúde geral. Primeiro, serão gravados os sons dos seus pulmões durante aproximadamente 20 segundos (3

repetições), com um microfone, como se fosse um estetoscópio, que está ligado a um computador portátil. Seguidamente, ser-lhe-á medido o peso e a altura numa balança. Depois, ser-lhe-á avaliada a força dos seus músculos da respiração e a capacidade respiratória, através de dois testes que consistem em inspirar e soprar para um equipamento. A avaliação da força dos seus músculos da coxa e braço realizar-se-ão de seguida através de um aparelho que se encosta à região do corpo em teste, é-lhe pedido que realize o máximo de força que conseguir e em breves segundos, o aparelho indica a força daquele músculo. Veremos também a sua tolerância ao exercício através do teste de sentar e levantar de uma cadeira. Ser-lhe-á também pedido para colocar um bocadinho de saliva para um copo (semelhante ao que utiliza quando realiza análises clínicas) para posterior análise. Mediremos também a quantidade de oxigénio no seu sangue e a sua frequência cardíaca através de um oxímetro (aparelho pequeno que se coloca no seu indicador e nos dá a informação desses valores em segundos). De seguida avaliaremos a sua frequência respiratória observando a sua região abdominal e mediremos a tensão arterial com um medidor de tensão arterial digital. Por último, ser-lhe-á pedido que responda a um questionário para avaliar o seu nível de atividades física e um outro para avaliar o impacto da sua doença no seu dia-a-dia. Se for cuidador de um doente com DPOC ou residir com o mesmo, ser-lhe-á também pedido que use um acelerómetro por 1 semana. Nenhum dos testes realizados provoca qualquer dor ou desconforto. A duração da avaliação será de aproximadamente 45 minutos e poderão ser realizadas em sua casa ou possivelmente nas instalações do Lab3R na Universidade de Aveiro, de acordo com a sua preferência.

No caso de ter DPOC terá também a possibilidade, no caso de experienciar uma exacerbação do seu estado de saúde, de realizar uma intervenção no sentido de melhorar o seu estado de saúde e ter a sua condição monitorizada durante 3 semanas.

Quais são os efeitos secundários, desvantagens e riscos se eu resolver participar?
Não existem efeitos secundários, desvantagens ou riscos de participar no estudo.

Quais são os possíveis benefícios se eu resolver participar?
Toda a informação clínica recolhida será fornecida aos participantes para que seja do seu conhecimento e, no caso de sofrer um agravamento dos sintomas, beneficiará de um acompanhamento semanal do seu estado de saúde prestado por um fisioterapeuta respiratório qualificado. Para além disso, a informação obtida neste estudo, através da sua participação, poderá ajudar a melhorar o conhecimento sobre a patogénese da DPOC e, a prevenção, diagnóstico precoce e gestão das exacerbações agudas, uma doença crónica que afeta cerca de 800.000 portugueses.

A minha participação será confidencial?
Toda a informação recolhida no decurso do estudo será mantida estritamente confidencial e mantido o anonimato. Os dados recolhidos serão salvaguardados com um código e palavra-passe, para que ninguém o/a possa identificar. Apenas os investigadores do projeto terão acesso aos seus dados.

O que acontecerá aos resultados do estudo?
Os resultados do estudo serão analisados e incorporados em Dissertações de Mestrado e Teses de Doutoramento e alguns serão publicados em Jornais Científicos. No entanto, em nenhum momento o Sr./Sra. será identificado/a. Se gostar de obter uma cópia de qualquer relatório ou publicação, por favor diga ao investigador com quem contactar.

Quem é que está a organizar e a financiar o estudo?
Este estudo foi financiado pelo Programa Operacional Competitividade e Internacionalização - COMPETE, através do Fundo Europeu de Desenvolvimento Regional - FEDER (POCI-01-0145-FEDER-016701), e pela Fundação para a Ciência e Tecnologia (PTDC/DTP-PIC/2284/2014). Este estudo foi também parcialmente apoiado pelo

COMPETE através do FEDER e da FCT através do projeto UID/BIM/04501/2013. O estudo decorre na Universidade de Aveiro em colaboração com o ACES e Centro Hospitalar do Baixo Vouga, Centro Hospitalar do Médio Ave, Hospital Pedro Hispano e Hospital Distrital da Figueira da Foz.

Contactos para mais informações sobre o estudo

Alda Marques (Investigadora Responsável)
Escola Superior de Saúde da Universidade de Aveiro,
Telefone 234 372 462
e-*mail: amarques@ua.pt*

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Appendix VII: Informed consent

Termo de Consentimento Livre e Esclarecido

Título do Projeto: "GENIAL – Marcadores genéticos e clínicos na trajetória da DPOC".

Nome do Investigador Principal: Prof. Doutora Alda Sofia Pires de Dias Marques

Por favor leia e assinale com uma cruz (X) os quadrados seguintes.

1. Eu confirmo que percebi a informação que me foi dada e tive a oportunidade de questionar e de me esclarecer. ☐

2. Eu percebo que a minha participação é voluntária e que sou livre de desistir, em qualquer altura, sem dar nenhuma explicação, sem que isso afete qualquer serviço de saúde ou qualquer outro que me é prestado. ☐

3. Eu compreendo que os dados recolhidos durante a investigação são confidenciais e que só os investigadores do projeto da Universidade de Aveiro têm acesso a eles. Portanto, dou autorização para que os mesmos tenham acesso a esses dados. ☐

4. Eu compreendo que os dados recolhidos durante o estudo podem ser utilizados para publicação em Revistas Científicas e usados noutras investigações, sem que haja qualquer quebra de confidencialidade. Portanto, dou autorização para a utilização dos dados para esses fins. ☐

5. Eu concordo então em participar no estudo. ☐

_____	_____	_____
Nome da pessoa	Data	Assinatura
_____	_____	_____
Nome do Investigador(a)	Data	Assinatura

Appendix VIII: Data collection protocol

Protocolo do Participante

Data___/___/____ Código _____
Dia Mês Ano

A. INFORMAÇÃO SÓCIO-DEMOGRÁFICA

A1. Género (1) [ ] feminino (2) [ ] masculino

A2. Data de Nascimento ___/___/____ (dia/mês/ano)

A3. Habilitações literárias:

(1) Não frequentou o sistema de ensino formal	[ ]	(2) Até ao 1º ciclo Ensino Básico (4º ano)	[ ]
(3) Até ao 2º ciclo Ensino Básico (6º ano)	[ ]	(4) Até ao 3º ciclo Ensino Básico (9º ano)	[ ]
(5) Até ao Ensino Secundário (12º ano)	[ ]	(6) Curso Médio	[ ]
(7) Ensino Superior	[ ]		

A4. Estado Civil:

(1) solteiro	[ ]	(2) casado	[ ]	(3) separado	[ ]
(4) divorciado	[ ]	(5) viúvo	[ ]	(6) união de facto	[ ]

A5. Ocupação habitual:

(1) Trabalho remunerado	[ ]	(2) Trabalho não remunerado	[ ]
(3) Estudante	[ ]	(4) Trabalho doméstico	[ ]
(5) Reformado	[ ]	(6) Desempregado (motivo saúde)	[ ]
(7) Desempregado (outros motivos)	[ ]	(8) Outro Especifique_____	[ ]

B. INFORMAÇÃO SUCINTA SOBRE SAÚDE

B1. Composição Corporal:

Altura: _____m Peso: _____kg % Massa Gorda: _____ IMC: _____

B2.a Tem hábitos tabágicos?

(1) NÃO [ ] (2) SIM [ ] Se SIM,
quantidade diária_____
desde que ano/idade _____

B2.b Se respondeu NÃO, tem história anterior de hábitos tabágicos?
(1) NÃO [ ] (2) SIM [ ] quantidade diária_____
número de anos _____

B3. Comorbilidades_____

B4. Medicação (prescrita pelo médico ou adquirida sem receita médica)_____

B5. Oxigenoterapia de longa duração

(1) NÃO [ ] (2) SIM [ ] se SIM, especificar
Litros/min_____Horas/dia_____Tipo_____

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B6. Ventilação não invasiva

(1) NÃO [ ] (2) SIM [ ] se SIM, especificar
Tipo_____Horas/dia_____

B7. Internamento hospitalar nos últimos 3 meses? e no último ano?

(1) NÃO [ ] (2) SIM Último ano [ ] se SIM, especificar o(s) motivo(s) e a duração
1_____; _____dias
2_____; _____dias
3_____; _____dias

B8. Episódio ida ao serviço de urgência últimos 3 meses? e no último ano?

(1) NÃO [ ] (2) SIM 3M [ ] se SIM, especificar o(s) motivo(s)
Último ano [ ] 1_____;
2_____;
3_____;

B9. No último ano, quantas crises de problemas respiratórios teve? (uma crise implica um agravamento dos sintomas respiratórios, para além da variabilidade normal de dia para dia, que implica procurar ajuda médica ou alterar a sua medicação)

Nenhuma crise [ ] 1 crise [ ] 2 crises [ ] 3 crises [ ] Outro____

B10. Dispneia (mMRC)

Grau 0 [ ] Grau 1 [ ] Grau 2 [ ] Grau 3 [ ] Grau 4 [ ]

Fonte The Global Initiative for Chronic Obstructive Lung Disease. (2015). Global Strategy for Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease - 2015 Update: The Global Initiative for Chronic Obstructive Lung Disease, Inc.

B11. Atividade física

a) Quantas vezes por semana, costuma realizar 20 minutos de atividade física intensa que o faz suar ou ficar ofegante? (por exemplo, jogging, levantamento de grandes pesos, cavar, aeróbica ou andar de bicicleta a um ritmo rápido)

3 vezes/semana [ ] 1 a 2 vezes/semana [ ] Nenhuma [ ]

b) Quantas vezes por semana, costuma realizar 30 minutos de atividade física moderada ou caminhada que aumenta a sua frequência cardíaca ou o faz respirar com mais dificuldade que o normal? (por exemplo, cortar a relva, transportar cargas leves, andar de bicicleta a um ritmo regular, ou jogar ténis em duplas)

> 5 vezes/semana [ ] 3 a 4 vezes/semana [ ]
1 a 2 vezes/semana [ ] Nenhuma [ ]

Sinais Vitais, SpO2 e outros sintomas

Frequência Respiratória:	Tensão Arterial:_____/_____
Saturação de Oxigénio (%):	Frequência Cardíaca:
Escala de BORG modificada (Dispneia)	Grau:
Escala de BORG modificada (Fadiga)	Grau:

Fonte: Borg, G., *Borg's Perceived Exertion and Pain Scales*. Champaign: IL: Human Kinetics1998, United States of America: Human Kinetics.

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Aquisição dos Sons Respiratórios Computorizados

Espontâneo [ ]
(0.4L/s – 0.7L/s) [ ]
(1.0L/s – 1.5L/s) [ ]

Traqueia [ ] Anterior direito [ ] Anterior esquerdo [ ] Lateral direito [ ]
Lateral esquerdo [ ] Posterior direito [ ] Posterior esquerdo [ ] Todos [ ]

Fonte: Rossi M, A.R.A.Sovijärvi, P. Piirilä V, L.,, F.Dalmasso, J.Vanderschoot. Environmental and subject conditions and breathing manoeuvres for respiratory sound recordings. European Respiratory Review. 2000;10:77: 611-5

Recolha de saliva [ ]

Zaragatoa [ ]

Espirometria [ ]

Valores	Teste 1	Teste 2	Teste 3	Teste 4	Teste 5
FEV_1%					
FVC					
FEV_1/FVC					

Fonte: Miller, M. R. (2005). Standardisation of spirometry. *European Respiratory Journal, 26*(2), 319-338. doi: 10.1183/09031936.05.00034805

Força Muscular Isométrica (dinamómetro)

Grupo Muscular		Teste 1	Teste 2	Teste 3
Extensores do joelho	D			
Handgrip	D			

D: Dominante

Fonte: O'Shea, S.D., N.F. Taylor, and J.D. Paratz, Measuring Muscle Strength for People With Chronic Obstructive Pulmonary Disease: Retest Reliability of Hand-Held Dynamometry. Archives of Physical Medicine and Rehabilitation, 2007. 88(1): p. 32-36

Avaliação da Força Músculos respiratórios

	Teste 1	Teste 2	Teste 3	Teste 4	Teste 5
PIM (cmH_2O)					
PEM (cmH_2O)					

Fonte: ATS/ERS Statement on respiratory muscle testing. American journal of respiratory and critical care medicine 166: 518-624, 2002.

Teste das 5 repetições sentar-levantar

	Teste 1	Teste 2	Teste 3	Teste 4	Teste 5
Tempo (s)					

Fonte: Jones, S. E., Kon, S. S., Canavan, J. L., Patel, M. S., Clark, A. L., Nolan, C. M., . . . Man, W. D. (2013). The five-repetition sit-to-stand test as a functional outcome measure in COPD. Thorax, 68(11), 1015-1020. doi: 10.1136/thoraxjnl-2013-203576

Teste de 1 minuto sentar-levantar

	Teste 1	Teste 2	Teste 3	Teste 4	Teste 5
Repetições					

Fonte: Ozalevli S, Ozden A, Itil O, Akkoclu A. Comparison of the sit-to-stand test with 6min walk test in patients with chronic obstructive pulmonary disease. Respiratory medicine. 2007;101(2):286-93.

Acelerómetro [ ] Par: _____

(apenas DPOC + cuidador)

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Teste de Avaliação da DPOC – CAT

Este questionário irá ajudá-lo a si e ao seu profissional de saúde a medir o impacto que a DPOC (Doença Pulmonar Obstrutiva Crónica) está a ter no seu bem-estar e no seu quotidiano. As suas respostas e a pontuação do teste podem ser utilizadas por si e pelo seu profissional de saúde para ajudar a melhorar a gestão da sua DPOC e a obter o máximo benefício do tratamento. Para cada um dos pontos a seguir, assinale com um (X) o quadrado que melhor o descreve presentemente. Certifique-se que seleciona apenas uma resposta para cada pergunta.

Por exemplo: Estou muito feliz 0 1 2 3 4 5 Estou muito triste

Nunca tenho tosse	0	1	2	3	4	5	**Estou sempre a tossir**
Não tenho nenhuma expetoração (catarro) no peito	0	1	2	3	4	5	**O meu peito está cheio de expetoração (catarro)**
Não sinto nenhum aperto no peito	0	1	2	3	4	5	**Sinto um grande aperto no peito**
Não sinto falta de ar ao subir uma ladeira ou um lance de escadas	0	1	2	3	4	5	**Quando subo uma ladeira ou um lance de escadas sinto bastante falta de ar**
Não sinto nenhuma limitação nas minhas atividades em casa	0	1	2	3	4	5	**Sinto-me muito limitado nas minhas atividades em casa**
Sinto-me confiante para sair de casa, apesar da minha doença pulmonar	0	1	2	3	4	5	**Não me sinto nada confiante para sair de casa, por causa da minha doença pulmonar**
Durmo profundamente	0	1	2	3	4	5	**Não durmo profundamente devido à minha doença pulmonar**
Tenho muita energia	0	1	2	3	4	5	**Não tenho nenhuma energia**

Fonte: George, F. H. M. (2013). *Diagnóstico e Tratamento da Doença Pulmonar Obstrutiva Crónica.*: Direção Geral de Saúde.

Muito obrigado pela sua colaboração.

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Escala de Ansiedade e Depressão Hospitalar - HADS

Este questionário foi construído para ajudar a saber como se sente. Pedimos-lhe que leia cada uma das perguntas e faça uma cruz (X) no espaço anterior à resposta que melhor descreve a forma como se tem sentido na última semana. Não demore muito tempo a pensar nas respostas. A sua reação imediata a cada questão será provavelmente mais correta do que uma resposta muito ponderada. Por favor, faça apenas uma cruz em cada pergunta.

(A)1. Sinto-me tenso/a ou nervoso/a:
- 3 □ Quase sempre
- 2 □ Muitas vezes
- 1 □ Por vezes
- 0 □ Nunca

(D)2. Ainda sinto prazer nas coisas de que costumava gostar:
- 0 □ Tanto como antes
- 1 □ Não tanto agora
- 2 □ Só um pouco
- 3 □ Quase nada

(A)3. Tenho uma sensação de medo, como se algo terrível estivesse para acontecer:
- 3 □ Sim e muito forte
- 2 □ Sim, mas não muito forte
- 1 □ Um pouco, mas não me aflige
- 0 □ De modo algum

(D)4. Sou capaz de rir e ver o lado divertido das coisas:
- 0 □ Tanto como antes
- 1 □ Não tanto como antes
- 2 □ Muito menos agora
- 3 □ Nunca

(A)5. Tenho a cabeça cheia de preocupações:
- 3 □ A maior parte do tempo
- 2 □ Muitas vezes
- 1 □ Por vezes
- 0 □ Quase nunca

(D)6. Sinto-me animado/a:
- 3 □ Nunca
- 2 □ Poucas vezes
- 1 □ De vez em quando
- 0 □ Quase sempre

(A)7. Sou capaz de estar descontraidamente sentado/a e sentir-me relaxado/a:
- 0 □ Quase sempre
- 1 □ Muitas vezes
- 2 □ Por vezes
- 3 □ Nunca

(D)8. Sinto-me mais lento/a, como se fizesse as coisas mais devagar:
- 3 □ Quase sempre
- 2 □ Muitas vezes
- 1 □ Por vezes
- 0 □ Nunca

(A)9. Fico de tal forma apreensivo/a (com medo), que até sinto um aperto no estômago:
- 0 □ Nunca
- 1 □ Por vezes
- 2 □ Muitas vezes
- 3 □ Quase sempre

(D)10. Perdi o interesse em cuidar do meu aspeto físico:
- 3 □ Completamente
- 2 □ Não dou a atenção que devia
- 1 □ Talvez cuide menos que antes
- 0 □ Tenho o mesmo interesse de sempre

(A)11. Sinto-me de tal forma inquieto/a que não consigo estar parado/a:
- 3 □ Muito
- 2 □ Bastante
- 1 □ Não muito
- 0 □ Nada

(D)12. Penso com prazer nas coisas que podem acontecer no futuro:
- 0 □ Tanto como antes
- 1 □ Não tanto agora
- 2 □ Bastante menos agora
- 3 □ Quase nunca

(A)13. De repente, tenho sensações de pânico:
- 3 □ Muitas vezes
- 2 □ Bastantes vezes
- 1 □ Por vezes
- 0 □ Quase nunca

(D)14. Sou capaz de apreciar um bom livro ou um programa de rádio ou televisão:
- 0 □ Muitas vezes
- 1 □ De vez em quando
- 2 □ Por vezes
- 3 □ Quase nunca

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Questionário do Hospital St. George na Doença Respiratória

Este questionário ajuda-nos a compreender até que ponto a sua dificuldade respiratória o/a perturba e afeta a sua vida. Usamo-lo para descobrir quais os aspetos da sua doença que lhe causam mais problemas. Interessa-nos saber o que sente e não o que os profissionais de saúde acham que serão os seus problemas.

Leia atentamente as instruções. Esclareça as dúvidas que tiver. Não perca muito tempo nas suas respostas.

Antes de preencher o questionário, assinale com um "**x**" a resposta que descreve melhor o seu estado de saúde atual:

Muito bom	Bom	Moderado	Mau	Muito mau
☐(1)	☐(2)	☐(3)	☐(4)	☐(5)

PARTE 1

Para cada uma das perguntas seguintes, assinale a resposta que melhor corresponde aos seus problemas respiratórios, nos últimos 3 meses**.**

Assinale um só quadrado para cada pergunta.	Maioria dos dias da semana	Vários dias da semana	Alguns dias no mês	Só com infeções respiratórias	Nunca
1. Durante os últimos 3 meses tossi:	□	□	□	□	□
2. Durante os últimos 3 meses tive expetoração:	□	□	□	□	□
3. Durante os últimos 3 meses tive falta de ar:	□	□	□	□	□
4. Durante os últimos 3 meses tive crises de pieira (chiadeira ou "gatinhos" no peito):	□	□	□	□	□
5. Durante os últimos 3 meses, quantas crises graves de problemas respiratórios teve?	Mais de 3 crises □	3 crises □	2 crises □	1 crise □	Nenhuma crise □
6. Quanto tempo durou a pior dessas crises? (passe para a pergunta 7 se não teve crises graves)	1 semana ou mais □	3 ou mais dias □	1 ou 2 dias □	Menos de 1 dia □	
7. Durante os últimos 3 meses, numa semana considerada como habitual, quantos dias bons (com poucos problemas respiratórios) teve?	Nenhum dia □	1 ou 2 dias □	3 ou 4 dias □	Quase todos os dias □	Todos os dias □
8. Se tem pieira (chiadeira, ou gatinhos" no peito), ela é pior de manhã?	Não □	Sim □			

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PARTE 2

Secção 1

Assinale um só quadrado para descrever a sua doença respiratória:	É o meu maior problema □	Causa-me muitos problemas □	Causa-me alguns problemas □	Não me causa nenhum problema □
Se tem ou já teve um trabalho pago, assinale uma das respostas:	A minha doença respiratória obrigou-me a parar de trabalhar □	A minha doença respiratória interfere (ou interferiu) com o meu trabalho normal ou já me obrigou a mudar de trabalho □	A minha doença respiratória não afeta (ou não afetou) o meu trabalho □	

Secção 2: Perguntas sobre as atividades que normalmente lhe têm provocado falta de ar nos últimos dias.

Assinale com "**x**" a resposta "concordo" ou "não concordo" de acordo com o seu caso:

	Concordo	Não concordo
Quando estou sentado(a) ou deitado(a)	□	□
A tomar banho ou a vestir-me	□	□
A caminhar dentro de casa	□	□
A caminhar em terreno plano	□	□
A subir um lanço de escadas	□	□
A subir ladeiras	□	□
A praticar desportos ou jogos que impliquem esforço físico	□	□

Secção 3: Mais algumas perguntas sobre a sua tosse e falta de ar nos últimos dias.

Assinale com "**x**" a resposta "concordo" ou "não concordo" de acordo com o seu caso:

	Concordo	Não concordo
A minha tosse causa-me dor	□	□
A minha tosse cansa-me	□	□
Falta-me o ar quando falo	□	□
Falta-me o ar quando me inclino para a frente	□	□
A minha tosse ou a falta de ar perturba o meu sono	□	□
Fico muito cansado(a) com facilidade	□	□

Secção 4: Perguntas sobre outros efeitos causados pela sua doença respiratória nos últimos dias. Assinale com "**x**" a resposta "concordo" ou "não concordo" de acordo com o seu caso:

	Concordo	Não concordo
A minha tosse ou falta de ar envergonham-me em público	□	□
A minha doença respiratória é um incómodo para a minha família, amigos ou vizinhos	□	□
Tenho medo ou receio ou mesmo pânico quando não consigo respirar	□	□
Sinto que não tenho controlo sobre a minha doença respiratória	□	□
Não espero melhoras da minha doença respiratória	□	□
A minha doença tornou-me fisicamente diminuído(a) ou inválido(a)	□	□
Fazer exercício é arriscado para mim	□	□
Tudo o que faço parece-me ser um esforço excessivo	□	□

Secção 5: Perguntas sobre a medicação para a sua doença respiratória. Caso não tenha medicação, passe para a secção 6. Assinale com "**x**" a resposta "concordo" ou "não concordo" de acordo com o seu caso:

	Concordo	Não concordo
A minha medicação não me está a ajudar muito	□	□
Tenho vergonha de tomar os medicamentos em público	□	□
A minha medicação provoca-me efeitos secundários desagradáveis	□	□
A minha medicação interfere muito com o meu dia a dia	□	□

Secção 6: As perguntas seguintes referem-se a atividades que podem ser afetadas pela sua doença respiratória. Assinale com "**x**" a resposta "concordo" se pelo menos uma parte da frase se aplica ao seu caso; se não, assinale "não concordo":

	Concordo	Não concordo
Levo muito tempo a lavar-me ou a vestir-me	□	□
Demoro muito tempo ou não consigo tomar banho ou um duche	□	□
Ando mais devagar que as outras pessoas, ou então tenho de parar para descansar	□	□
Demoro muito tempo com tarefas como o trabalho de casa, ou então tenho de parar para descansar	□	□
Quando subo um lanço de escadas, ou vou muito devagar ou tenho de parar para descansar	□	□
Se estou apressado ou se caminho mais depressa, tenho de parar ou diminuir o passo	□	□

	Concordo	Não concordo
Por causa da minha doença respiratória, tenho dificuldade em fazer coisas como: subir ladeiras, carregar pesos quando subo escadas, tratar do jardim ou do quintal, arrancar ervas, dançar, jogar à bola	□	□
Por causa da minha doença respiratória, tenho dificuldade em fazer coisas como: carregar grandes pesos, cavar o jardim ou o quintal, caminhar depressa (8 quilómetros/hora), jogar ténis ou nadar	□	□
Por causa da minha doença respiratória, tenho dificuldade em fazer coisas como: trabalho manual pesado, correr, andar de bicicleta, nadar com velocidade, ou praticar desportos muito cansativos	□	□

Secção 7: Gostaríamos de saber como é que a sua doença respiratória habitualmente afeta o seu dia a dia. Assinale com "**x**" a resposta "concordo" ou "não concordo".
(Não se esqueça que "concordo" só se aplica quando não puder fazer a atividade devido à sua doença respiratória). Assinale todas as perguntas que se aplicam a si:

	Concordo	Não concordo
Não sou capaz de praticar desportos ou jogos que impliquem esforço físico	□	□
Não sou capaz de sair de casa para me divertir	□	□
Não sou capaz de sair de casa para fazer compras	□	□
Não sou capaz de fazer o trabalho de casa	□	□
Não sou capaz de sair da cama ou da cadeira	□	□

Assinale com "**x**" (só um) a resposta que melhor define a forma como é afetado(a) pela sua doença respiratória:

Não me impede de fazer nenhuma das coisas que eu gostaria de fazer (0)	Impede-me de fazer uma ou duas coisas que eu gostaria de fazer (1)	Impede-me de fazer muitas das coisas que eu gostaria de fazer (2)	Impede-me de fazer tudo o que eu gostaria de fazer (3)
□	□	□	□

Muito obrigado pela sua colaboração.